PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 7/01, 15/86, A61K 39/275, 39/295 | A1

(11) International Publication Number: WO 98/04684

(43) International Publication Date: 5 February 1998 (05.02.98)

(21) International Application Number: PCT/US97/12212

(22) International Filing Date: 25 July 1997 (25.07.97)

(30) Priority Data:
08/686,968 25 July 1996 (25.07.96) US

(60) Parent Application or Grant
(63) Related by Continuation
US Not furnished (CIP)
Filed on Not furnished

(71) Applicant (*for all designated States except US*): SYNTRO CORPORATION [US/US]; 9669 Lackman Road, Lenexa, KS 66219 (US).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): COCHRAN, Mark, D. [US/US]; 4506 Horizon Drive, Carlsbad, CA 92008 (US). JUNKER, David, E. [US/US]; 6901 Galewood Street, San Diego, CA 92120 (US).

(74) Agent: WHITE, John, P.; Cooper & Dunham LLP, 1185 Avenue of the Americas, New York, NY 10068 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: RECOMBINANT SWINEPOX VIRUS

(57) Abstract

This invention provides a recombinant swinepox virus comprising a foreign DNA inserted into a swinepox virus genome, wherein the foreign DNA is inserted into a) an AccI site within a region corresponding to a 3.2 Kb HindIII to BglII subfragment of the HindII M fragment and b) an EcoRI site within a region corresponding to a 3.2 Kb subfragment of the HindIII K fragment which contains both a HindIII and EcoRI site, of the swinepox virus genome and is capable of being expressed in a host cell into which the virus is introduced. The invention further provides vaccines and methods of immunization of the recombinant swinepox virus.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

RECOMBINANT SWINEPOX VIRUS

Within this application several publications are referenced by arabic numerals within parentheses. Full citations for these publications may be found at the end of the specification immediately preceding the claims. The disclosures of these publications are hereby incorporated by reference into this application.

BACKGROUND OF THE INVENTION

Swinepox virus (SPV) belongs to the family *Poxviridae*. Viruses belonging to this group are large, double-stranded DNA viruses that characteristically develop in the cytoplasm of the host cell. SPV is the only member of the genus *Suipoxvirus*. Several features distinguish SPV from other poxviruses. SPV exhibits species specificity (18) compared to other poxviruses such as vaccinia which exhibit a broad host range. SPV infection of tissue culture cell lines also differs dramatically from other poxviruses (24). It has also been demonstrated that SPV does not exhibit antigenic cross-reactivity with vaccinia virus and shows no gross detectable homology at the DNA level with the ortho, lepori, avi or entomopox virus groups (24). Accordingly, what is known and described in the prior art regarding other poxviruses does not pertain *a priori* to swinepox virus.

SPV is only mildly pathogenic, being characterized by a self-limiting infection with lesions detected only in the skin and regional lymph nodes. Although the SPV infection is quite limited, pigs which have recovered from SPV are refractory to challenge with SPV, indicating development of active immunity (18).

The present invention concerns the use of SPV as a vector for the delivery of vaccine antigens and therapeutic agents to swine. The following properties of SPV support this rationale: SPV is only mildly pathogenic in swine, SPV is species specific, and SPV elicits a protective immune response. Accordingly, SPV is an excellent candidate for a viral vector delivery system, having little intrinsic risk which must be balanced against the benefit contributed by the vector's vaccine and therapeutic properties.

The prior art for this invention stems first from the ability to clone and analyze DNA while in bacterial plasmids. The techniques that are available are detailed for the most part in Maniatis *et al.*, 1983 and Sambrook *et al.*, 1989. These publications teach state of the art general recombinant DNA techniques.

Among the poxviruses, five (vaccinia, fowlpox, canarypox, pigeon, and raccoon pox) have been engineered, previous to this disclosure, to contain foreign DNA sequences. Vaccinia virus has been used extensively to vector foreign genes (25) and is the subject of U.S. Patents 4,603,112 and 4,722,848. Similarly, fowlpox has been used to vector foreign genes and is the subject of several patent applications EPA 0 284 416, PCT WO 89/03429, and PCT WO 89/12684. Raccoon pox (10) and Canarypox (31) have been utilized to express antigens from the rabies virus. These examples of insertions of foreign genes into poxviruses do not include an example from the genus *Suipoxvirus*. Thus, they do not teach methods to genetically engineer swinepox viruses, that is, where to make insertions and how to get expression in swinepox virus.

The idea of using live viruses as delivery systems for antigens has a very long history going back to the first live virus vaccines. The antigens delivered were not foreign but were naturally expressed by the live virus in the vaccines. The use of viruses to deliver foreign antigens in the modern sense became obvious with the recombinant vaccinia virus studies. The vaccinia virus was the vector and various antigens from other disease causing viruses were the foreign antigens, and the vaccine was created by genetic engineering. While the concept became obvious with these disclosures, what was not obvious was the answer to a more practical question of what makes the best candidate virus vector. In answering this question, details of the pathogenicity of the virus, its site of replication, the kind of immune response it elicits, the potential it has to express foreign antigens, its suitability for genetic engineering, its probability of being licensed by regulatory agencies, etc, are all factors in the selection. The prior art does not teach these questions of utility.

The prior art relating to the use of poxviruses to deliver therapeutic agents relates to the use of a vaccinia virus to deliver interleukin-2 (12). In this case, although the interleukin-2 had an attenuating effect on the vaccinia vector, the host did not demonstrate any therapeutic benefit.

The therapeutic agent that is delivered by a viral vector of the present invention must be a biological molecule that is a by-product of swinepox virus replication. This limits the therapeutic agent in the first analysis to either DNA, RNA or protein. There are examples of therapeutic agents from each of these classes of compounds in the form of anti-sense DNA, anti-sense RNA (16), ribozymes (34), suppressor tRNAs (2), interferon-

inducing double stranded RNA and numerous examples of protein therapeutics, from hormones, e.g., insulin, to lymphokines, e.g., interferons and interleukins, to natural opiates. The discovery of these therapeutic agents and the elucidation of their structure and function does not make obvious the ability to use them in a viral vector delivery system.

SUMMARY OF THE INVENTION

This invention provides a recombinant swinepox virus comprising a foreign DNA inserted into a swinepox virus genome, wherein the foreign DNA is inserted into an EcoRI site within a region corresponding to a 3.2 Kb subfragment of the HindIII K fragment which contains both a HindIII and an EcoRI site, of the swinepox virus genome and is capable of being expressed in a host cell into which the virus is introduced.

This invention provides a recombinant swinepox virus comprising a foreign DNA inserted into a swinepox virus genome, wherein the foreign DNA is inserted into a) an AccI site within a region corresponding to a HindIII to BglII subfragment of the HindIII M fragment and b) an EcoRI site within a region corresponding to a 3.2 Kb subfragment of the HindIII K fragment which contains both a HindIII and EcoRI site, of the swinepox virus genome and is capable of being expressed in a host cell into which the virus is introduced.

The invention further provides vaccines and methods of immunization of the recombinant swinepox virus.

BRIEF DESCRIPTION OF THE INVENTION

Figures 1A - 1B:

Show a detailed diagram of SPV genomic DNA (Kasza strain) including the unique long and Terminal repeat (TR) regions. A restriction map for the enzyme *Hind*III is indicated (23). Fragments are lettered in order of decreasing size. Note that the terminal repeats are greater than 2.1 kb but less than 9.7 kb in size.

Figures 2A - 2C:

Show the homology which exists between the 515.85.1 ORF and the Vaccinia virus 01L ORF. Figure 2A shows two maps: The first line of Figure 2A is a restriction map of the SPV *Hind*III M fragment and the second is a restriction map of the DNA insertion in plasmid 515-85.1. The location of the 515-85.1 [VV 01L-like] ORF is also indicated on the map. The locations of the DNA sequences shown in Figures 2B and 2C are indicated below the map by heavy bars in Figure 2A. Figure 2B shows the homology between the VV 01L ORF and the 515-85.1 ORF at their respective N-termini. Figure 2C shows the homology between the VV 01L ORF and the 515-85.1 ORF at their respective C-termini.

Figures 3A - 3C:

Show a description of the DNA insertion in Homology Vector 520-17.5. Figure 3A contains a diagram showing the orientation of DNA fragments assembled in plasmid 520-17.5 and table indicating the origin of each fragment. Figure 3B shows the sequences located at each of the junctions A and B between fragments, and Figure 3C shows the sequences located at Junctions C and D. Figures 3B and 3C further describe the restriction sites used to generate each

fragment as well as the synthetic linker sequences which were used to join the fragments are described for each junction. The synthetic linker sequences are underlined by a heavy bar. The location of several gene coding regions and regulatory elements are also given. The following two conventions are used: numbers in parenthesis () refer to amino acids, and restriction sites in brackets [] indicate the remnants of sites which were destroyed during construction. The following abbreviations are used, swinepox virus (SPV), early promoter 1 (EP1), late promoter 2 (LP2), lactose operon Z gene (lacZ), and *Escherichia coli (E. coli)*.

Figures 4A- 4D:

Show a detailed description of the DNA insertion in Homology Vector 538-46.16. Figure 4A contains a diagram showing the orientation of DNA fragments assembled in plasmid 538-46.16 and a table indicating the origin of each fragment. Figure 4B shows the sequences located at Junctions A and B between fragments, Figure 4C shows sequences located at Junction C and Figure 4D shows sequences located at Junctions D and E. Figures 4B to 4D also describe the restriction sites used to generate each fragment as well as the synthetic linker sequences which were used to join the fragments are described for each junction. The synthetic linker sequences are underlined by a heavy bar. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parenthesis () refer to amino acids, and restriction sites in brackets [] indicate the remnants of sites which were destroyed during construction. The following abbreviations are used, swinepox virus (SPV), pseudorabies virus (PRV), g50 (gD), glycoprotein 63 (g63), early promoter 1 (EP1),

late promoter 1 (LP1), late promoter 2 (LP2), lactose operon Z gene (lacZ), and *Escherichia coli* (*E. coli*).

Figures 5A - 5D:

Show a detailed description of Swinepox Virus S-PRV-013 and the DNA insertion in Homology Vector 570-91.64. Figure 5A contains a diagram showing the orientation of DNA fragments assembled in plasmid 570-91.64 and a table indicating the origin of each fragment. Figure 5B shows the sequences located at Junctions A and B between fragments, Figure 5C shows the sequences located at Junction C, and Figure 5D shows the sequences located at Junctions D and E . The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction in Figures 5B to 5D. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restriction sites in brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), pseudorabies virus (PRV), *Escherichia coli* (*E. coli*), pox synthetic late promoter 1 (LP1), pox synthetic late promoter 2 early promoter 2 (LP2EP2), gIII (gC) base pairs (BP).

Figure 6:

Map showing the 5.6 kilobase pair HindIII M swinepox virus genomic DNA fragment. Open reading frames (ORF) are shown with the number of amino acids coding in each open reading frame. The swinepox virus ORFs show significant sequence identities to

the vaccinia virus ORFs and are labeled with the vaccinia virus nomenclature (56 and 58). The I4L ORF (SEQ ID NO: 196) shows amino acid sequence homology to ribonucleotide reductase large subunit (57), and the 01L ORF (SEQ ID NO: 193) shows amino acid sequence homology to a leucine zipper motif characteristic of certain eukaryotic transcriptional regulatory proteins (13). The BglII site in the I4L ORF and the AccI site in the 01L ORF are insertion sites for foreign DNA into non-essential regions of the swinepox genome. The homology vector 738-94.4 contains a deletion of SPV DNA from nucleotides 1679 to 2452 (SEQ ID NO: 189). The black bar at the bottom indicates regions for which the DNA sequence is known and references the SEQ ID NOs: 189 and 195. Positions of restriction sites AccI, BglII, and HindIII are shown. I3L ORF (SEQ ID NO: 190), I2L ORF (SEQ ID NO: 191) and E1OR ORF (SEQ ID NO: 194) are shown. SEQ ID NO 221 contains the complete 5785 base pair sequence of the HindIII M fragment. Open reading frames within the SPV HindIII M fragment are the partial I4L ORF (445 AA; Nucl 2 to 1336); I3L ORF (275 AA; Nucl 1387 to 2211); I2L ORF (75 AA; Nucl 2215 to 2439); I1L ORF (313 AA; Nucl 2443 to 3381); O1L ORF (677 AA; Ncl 3520 to 5550); partial E1OR ORF (64 AA; Nucl 5787 to 5596).

Figures 7A - 7D:

Show a detailed description of Swinepox Virus S-SPV-015 and the DNA insertion in Homology Vector 727-54.60. Figure 7A contains a diagram showing the orientation of DNA fragments assembled in plasmid 727-54.60 and a table indicating the origin of each fragment. Figure 7B shows the sequences located at Junctions A and B between fragments, Figure 7C shows the sequences located at Junction C, and Figure 7D shows the sequences located at Junctions D and E.

The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction in Figures 7B to 7D. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restriction sites in brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), pseudorabies virus (PRV), *Escherichia coli (E. coli)*, pox synthetic late promoter 1 (LP1), pox synthetic late promoter 2 early promoter 2 (LP2EP2), glycoprotein B (gB), base pairs (BP).

Figures 8A-8D:

Detailed description of Swinepox Virus S-SPV-042 and the DNA insertion in Homology Vector 751-07.A1. Diagram showing the orientation of DNA fragments assembled in plasmid 751-07.A1. The origin of each fragment is indicated in the table. The sequence located at each of the junctions between fragments is also shown. The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction. Figures 8A-8D show the sequences located at Junction A (SEQ ID NOS: 197), (SEQ ID NO: 198), C (SEQ ID NO: 199), D (SEQ ID NO: 200) and E (SEQ ID NO: 201) between fragments and the sequences located at the junctions. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restriction sites in brackets, [], indicate the remnants of sites which

are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), chicken myelomonocytic growth factor (cMGF), Escherichia coli (E. coli), pox synthetic late promoter 1 (LP1), pox synthetic late promoter 2 early promoter 2 (LP2EP2), polymerase chain reaction (PCR), base pairs (BP).

Figures 9A-9D:

Detailed description of Swinepox Virus S-SPV-043 and the DNA insertion in Homology Vector 751-56.A1. Diagram showing the orientation of DNA fragments assembled in plasmid 751-56.A1. The origin of each fragment is indicated in the table. The sequences located at each of the junctions between fragments is also shown. Figures 9A-9D show the sequences located at Junction A (SEQ ID NOS: 202), (SEQ ID NO: 203), C (SEQ ID NO: 204), D (SEQ ID NO: 205) and E (SEQ ID NO: 206) between fragments and the sequences located at the junctions. The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restriction sites in brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV),chicken interferon (cIFN), Escherichia coli (E. coli), pox synthetic late promoter 1 (LP1), pox synthetic late promoter 2 early promoter 2 (LPE2EP2), polymerase chain reaction (PCR), base pairs (BP).

Figure 10A-10D:

Detailed description of Swinepox Virus S-SPV-037 and the DNA insertion in Homology Vector 752-22.1.

Diagram showing the orientation of DNA fragments assembled in plasmid 752-22.1. The origin of each fragment is indicated in the table. The sequences located at each of the junctions between fragments is also shown. Figures 10A-10D show the sequences located at Junction A (SEQ ID NOS: 207), (SEQ ID NO: 208), C (SEQ ID NO: 209), and D (SEQ ID NO: 210) between fragments and the sequences located at the junctions. The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restrictions sites in brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), Escherichia coli (E. coli), pox synthetic late promoter 2 early promoter 2 (LP2EP2), polymerase chain reaction (PCR), base pairs (BP).

Figures 11A-11B:

Figure 11A: Restriction Endonuclease Map and Open Reading Frames in the SPV HindIII N fragment and part of SPV HindIII M fragment. Insertions of a foreign gene into a non-essential site of the swinepox virus Hind III N and Hind III M genomic DNA include the EcoR V site (S-SPV-060), SnaB I site (S-SPV-061), Bgl II site in Hind III N (S-SPV-062), and the Bgl II site in Hind III M (S-SPV-047). Insertions of a foreign gene into the I7L ORF (SEQ ID NO. 230) and I4L ORF (SEQ ID NO. 231) indicates that the sequence of the entire open reading frame is non-essential for replication of the swinepox virus and suitable for insertion of foreign genes.

Additional sites for insertion of foreign genes include, but are not limited to the two Hind III sites, Ava I site, and the BamHI site.

Figure 11B: Restriction Endonuclease Map and Open Reading Frames in the SPV Hind III K genomic fragment. Insertion of a foreign gene into a non-essential site of the swinepox virus Hind III K genomic DNA includes, but is not limited to the unique EcoR I site (S-SPV-059). Three open reading frames (ORFs) were identified within an approximately 3.2 kB region (SEQ ID NO. 1) of the approximately 6.7 kb SPV HindIII K fragment. Insertions of a foreign DNA into a unique EcoRI site within the SPV HindIII K genomic fragment indicates that the sequence is non-essential for replication of the swinepox virus and suitable for insertion of foreign genes.The unique EcoRI site is located between the 77.2 kd protein ORF and the T5 protein ORF in an intergenic region indicating that the intergenic region contains suitable sites for insertion of foreign DNA. Also identified are the 77.2 kd protein)RF (SEQ ID NO:3) and the T5 protein ORF (SEQ ID NO. 4) and an ORF of unknown function (SEQ ID NO. 2) which are suitable sites for insertion of a foreign DNA. The SPV 77.2 kd protein ORF (SEQ ID NO. 3) has amino acid sequence homology to rabbit fibroma virus (RFV) 77.2 kd protein. The SPV T5 protein ORF has amino acid sequence homology to rabbit fibroma virus (RFV) T5 protein. The identified open reading frames are within an approximately 3141 base pair segment of the SPV Hind III K fragment (SEQ ID NO. 1). The remaining approximately 3500 base pairs of the SPV Hind III K fragment has been sequenced previously (R.F. Massung, et al. Virology **197**, 511-528 (1993)).

Figures 12A-12C:

Detailed description of Swinepox Virus S-SPV-047 and the DNA insertion in Homology Vector 779-94.31. Diagram showing the orientation of DNA fragments assembled in plasmid 779-94.31. The origin of each fragment is indicated in the table. The sequences located at each of the junctions between fragments is also shown. Figures 12A-12C show the sequences located at Junction A (S:), , C , D , and E between fragments and the sequences located at the junctions. The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restrictions sites in brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), pseudorabies virus (PRV), Escherichia coli (E. coli), pox synthetic late promoter 2 early promoter 2 (LP2EP2), pox synthetic late promoter 1 (LP1), base pairs (BP).

Figures 13A-13D:

Detailed description of Swinepox Virus S-SPV-052 and the DNA insertion in Homology Vector 789-41.7. Diagram showing the orientation of DNA fragments assembled in plasmid 789-41.7. The origin of each fragment is indicated in the table. The sequences located at each of the junctions between fragments is also shown. Figures 13A-13D show the sequences located at Junction A, B, C , D , E , and F between fragments and the sequences located at the junctions. The restriction sites used to generate

each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restrictions sites in brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), pseudorabies virus (PRV), Escherichia coli (E. coli), pox synthetic late promoter 2 early promoter 2 (LP2EP2), pox synthetic early promoter 1 late promoter 2 (EP1LP2), pox synthetic late promoter 1 (LP1), base pairs (BP).

Figures 14A-14D:

Detailed description of Swinepox Virus S-SPV-053 and the DNA insertion in Homology Vector 789-41.27. Diagram showing the orientation of DNA fragments assembled in plasmid 789-41.27. The origin of each fragment is indicated in the table. The sequences located at each of the junctions between fragments is also shown. Figures 14A-14D show the sequences located at Junction A, B, C , D , E , F , and G between fragments and the sequences located at the junctions. The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restrictions sites in brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), pseudorabies virus (PRV), Escherichia coli (E. coli), pox synthetic late

promoter 2 early promoter 2 (LP2EP2), pox synthetic early promoter 1 late promoter 2 (EP1LP2), pox synthetic late promoter 1 (LP1), base pairs (BP).

Figures 15A-15D:

Detailed description of Swinepox Virus S-SPV-054 and the DNA insertion in Homology Vector 789-41.47. Diagram showing the orientation of DNA fragments assembled in plasmid 789-41.47. The origin of each fragment is indicated in the table. The sequences located at each of the junctions between fragments is also shown. Figures 15A-15D show the sequences located at Junction A, B, C (SEQ ID NO:), D , E , F , and G between fragments and the sequences located at the junctions. The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restrictions sites in brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), pseudorabies virus (PRV), Escherichia coli (E. coli), pox synthetic early promoter 1 late promoter 2 (EP1LP2), pox synthetic late promoter 1 (LP1), base pairs (BP).

Figures 16A-16E:

Detailed description of Swinepox Virus S-SPV-055 and the DNA insertion in Homology Vector 789-41.73. Diagram showing the orientation of DNA fragments assembled in plasmid 789-41.73. The origin of each fragment is indicated in the table. The sequences

located at each of the junctions between fragments is also shown. Figures 16A-16E show the sequences located at Junction A, B, C , D , E , F , G , and H between fragments and the sequences located at the junctions. The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restrictions sites in brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), pseudorabies virus (PRV), Escherichia coli (E. coli), pox synthetic late promoter 2 early promoter 2 (LP2EP2), pox synthetic early promoter 1 late promoter 2 (EP1LP2), pox synthetic late promoter 1 (LP1), base pairs (BP).

DETAILED DESCRIPTION OF THE INVENTION

This invention provides a recombinant swinepox virus comprising a foreign DNA inserted into a swinepox virus genome, wherein the foreign DNA is inserted into an EcoRI site within a region corresponding to a 3.2 Kb subfragment of the HindIII K fragment which contains both a HindIII and an EcoRI site, of the swinepox virus genome and is capable of being expressed in a host cell into which the virus is introduced.

This invention provides a recombinant swinepox virus comprising a foreign DNA inserted into a swinepox virus genome, wherein the foreign DNA is inserted into a) an AccI site within a region corresponding to a HindIII to BglII subfragment of the larger HindIII M fragment and b) an EcoRI site within a region corresponding to a 3.2 Kb subfragment of the HindIII K fragment which contains both a HindIII and EcoRI site, of the swinepox virus genome and is capable of being expressed in a host cell into which the virus is introduced.

In another embodiment the open reading frame encodes a B18R gene. In another embodiment the open reading frame encodes a B4R gene. In another embodiment the open reading frame encodes swinepox homologue of the 77.2 kD protein gene. In another embodiment the open reading frame encodes swinepox homologue of the T5 protein gene.

In another embodiment the foreign DNA sequence is inserted within a EcoRV restriction endonuclease site within the approximately 2 kB HindIII to BamHI subfragment of the swinepox virus genome. In another embodiment the foreign DNA sequence is inserted within a SnaBI restriction endonuclease site within the

approximately 2.0 kB HindIII to BamHI subfragment of the swinepox virus genome.

In another embodiment the foreign DNA sequence is inserted within an approximately 1.2 kB BamHI to HindIII subfragment of the HindIII N fragment of the swinepox virus genome. In another embodiment the foreign DNA sequence is inserted into an open reading frame within an approximately 1.2 kB BamHI to HindIII subfragment of the HindIII N fragment of the swinepox virus genome. In another embodiment the foreign DNA sequence is inserted into an open reading frame which encodes a I4L gene. In another embodiment the foreign DNA sequence is inserted within a BglII restriction endonuclease site within the approximately 1.2 kB BamHI to HindIII subfragment of the swinepox virus genome.

In another embodiment the recombinant swinepox virus contains the foreign DNA sequence inserted within an approximately 3.6 kB larger HindIII to BglII subfragment of the HindIII M fragment of the swinepox virus genomic DNA. In another embodiment the foreign DNA sequence is inserted into an open reading frame within an approximately 3.6 kB larger HindIII to BglII subfragment of the HindIII M fragment of the swinepox virus genomic DNA. In another embodiment the open reading frame encodes a I4L gene.

In one embodiment the foreign DNA sequence of the recombinant swinepox virus is inserted within a non-essential Open Reading Frame (ORF) of the HindIII M fragment. Example of ORF's include, but are not limited to: I4L, I2L, 01L, and E10L.

In another embodiment the recombinant swinepox virus further comprises a foreign DNA sequence inserted into an open reading frame encoding swinepox virus thymidine

kinase. In one embodiment the foreign DNA sequence is inserted into a *Nde*I site located within the open reading frame encoding the swinepox virus thymidine kinase.

For purposes of this invention, "a recombinant swinepox virus capable of replication" is a live swinepox virus which has been generated by the recombinant methods well known to those of skill in the art, e.g., the methods set forth in HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV in Materials and Methods and has not had genetic material essential for the replication of the recombinant swinepox virus deleted.

For purposes of this invention, "an insertion site which is not essential for replication of the swinepox virus" is a region or a region which corresponds to a specific fragment in the swinepox viral genome where a sequence of DNA is not necessary for viral replication, for example, complex protein binding sequences, sequences which code for reverse transcriptase or an essential glycoprotein, DNA sequences necessary for packaging, etc.

For purposes of this invention, a "promoter" is a specific DNA sequence on the DNA molecule to which the foreign RNA polymerase attaches and at which transcription of the foreign RNA is initiated.

For purposes of this invention, an "open reading frame" is a segment of DNA which contains codons that can be transcribed into RNA which can be translated into an amino acid sequence and which does not contain a termination codon.

The invention further provides a foreign DNA sequence or foreign RNA which encodes a polypeptide. Preferably, the polypeptide is antigenic in the animal. Preferably, this antigenic polypeptide is a linear polymer of more than 10

amino acids linked by peptide bonds which stimulates the animal to produce antibodies.

The S-SPV-003 swinepox virus has been deposited pursuant to the Budapest Treaty on the International Deposit of Microorganisms for the Purposes of Patent Procedure with the Patent Culture Depository of the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852 U.S.A. under ATCC Accession No. VR 2335.

For purposes of this invention, a "polypeptide which is a detectable marker" includes the bimer, trimer and tetramer form of the polypeptide. *E. coli* ß-galactosidase is a tetramer composed of four polypeptides or monomer sub-units.

Foreign DNA which code for a polypeptide include but are not limited to: feline leukemia virus surface protein, feline leukemia virus transmembrane protein, feline leukemia virus gag, feline leukemia virus transmembrane protease, feline immunodeficiency virus gag/protease, feline immunodeficiency virus envelope, feline leukemia virus gag/protease, feline leukemia virus envelope, canine parvovirus VP2, canine parvovirus VP1/2, bovine cytokine interleukin-12 protein 35, bovine cytokine interleukin-12 protein 40, Bovine Respiratory Syncytial Virus glycoprotein G, Newcastles Disease fusion, Infectious Rhinotracheitis Virus glycoprotein D, Canine Distemper Virus fusion, Canine Distemper Virus Hemagluttinin, DV HA, Bovine Viral Diarrhea Virus type 1 glycoprotein 45, Bovine Viral Diarrhea Virus type 1 glycoprotein 48, Bovine Viral Diarrhea Virus type 1 glycoprotein 53, Bovine Viral Diarrhea Virus type 2 glycoprotein 53.

The present invention further provides a recombinant swinepox virus in which the foreign DNA encodes an antigenic polypeptide is: Swine Influenza Virus hemagglutin, Swine Influenza Virus neurominidase,Swine Influenza Virus matrix, Swine Influenza Virus nuceloprotein, African Swine Fever Virus or *Mycoplasma hyopneumoniae*. Preferred embodiments of such virus are designated S-SPV-121, and S-SPV-122.

The present invention further provides a recombinant swinepox virus in which the foreign DNA encodes an antigenic polypeptide is: cytokine is chicken macrophage migration inhibitory factor (cMIF), chicken myelomonocytic growth factor (cMGF) or chicken interferon (cIFN). Preferred embodiments of such virus are designated S-SPV-068, and S-SPV-105.

The present invention further provides a recombinant swinepox virus in which the foreign DNA encodes an antigenic polypeptide is: porcine reproductive and respiratory syndrome virus (PRRS) ORF2, ORF3, ORF4, ORF5, ORF6 and ORF7, pseudorabies gB, gD, gI. Preferred embodiments of such virus are designated S-SPV-076, S-SPV-079, S-SPV-090, S-SPV-084,S-SPV-091, S-SPV-092, S-SPV-093, S-SPV-094, S-SPV-095.

The present invention further provides a recombinant swinepox virus in which the foreign DNA encodes an antigenic polypeptide is: Infectious bovine rhinotracheitis virus glycoprotein B, glycoprotein D and glycoprotein I, bovine respiratory syncytial virus attachment protein (BRSV G), bovine respiratory syncytial virus fusion protein (BRSV F), bovine respiratory syncytial virus nucleocapsid protein (BRSV N). Preferred embodiments of such virus are designated S-SPV-109, S-

SPV-110, S-SPV-111, S-SPV-113, S-SPV-115, S-SPV-119, S-SPV-112.

The present invention further provides a recombinant swinepox virus in which the foreign DNA encodes an antigenic polypeptide is: bovine viral diarrhea virus (BVDV) glycoprotein 48 or glycoprotein 53. A Preferred embodiment of such a virus is designated S-SPV-099.

The present invention further provides a recombinant swinepox virus in which the foreign DNA encodes an antigenic polypeptide is: feline immunodeficiency virus gag/protease and envelope, feline leukemia virus gag/protease and envelope. Preferred embodiments of such viruses are designated: S-SPV-106, S-SPV-089, S-SPV-100, S-SPV-107, S-SPV-108.

The present invention further provides a recombinant swinepox virus in which the foreign DNA encodes an antigenic polypeptide is: canine parvovirus VP2 and VP1/2. Preferred embodiments of such viruses are designated: S-SPV-114, S-SPV-116, S-SPV-117, S-SPV-118.

The present invention provides a recombinant swinepox virus comprising a foreign DNA inserted into the swinepox virus genomic DNA, wherein the one or more foreign DNAs are inserted within each of the HindIII K fragment of the swinepox virus genomic DNA and within the HindIII M fragment of the swinepox virus genomic DNA and is capable of being expressed in a swinepox virus infected host cell. Preferred embodiments of such viruses are designated: S-SPV-127, S-SPV-128, S-SPV-131, and S-SPV-132.

The present invention provides a recombinant swinepox virus comprising a foreign DNA inserted into the swinepox virus genomic DNA, wherein the one or more foreign DNAs which encode a fusion protein are inserted within each of the HindIII K fragment of the swinepox virus genomic DNA and within the HindIII M fragment of the swinepox virus genomic DNA and is capable of being expressed in a swinepox virus infected host cell. Preferred embodiments of such viruses are designated: S-SPV-130.

The invention further provides a recombinant swinepox virus capable of replication which contains foreign DNA encoding an antigenic polypeptide which is or is from pseudorabies virus (PRV) g50 (gD), pseudorabies virus (PRV) gII (gB), Pseudorabies virus (PRV) gIII (gC), pseudorabies virus (PRV) glycoprotein H, pseudorabies virus (PRV) glycoprotein E, Transmissible gastroenteritis (TGE) glycoprotein 195, Transmissible gastroenteritis (TGE) matrix protein, swine rotavirus glycoprotein 38, swine parvovirus capsid protein, *Serpulina hydodysenteriae* protective antigen, Bovine Viral Diarrhea (BVD) glycoprotein 55, Newcastle Disease Virus (NDV) hemagglutinin-neuraminidase, swine flu hemagglutinin or swine flu neuraminidase. Preferably, the antigenic polypeptide is Pseudorabies Virus (PRV) g50 (gD). Preferably, the antigenic protein is Newcastle Disease Virus (NDV) hemagglutinin-neuraminidase.

The invention further provides a recombinant swinepox virus capable of replication which contains foreign DNA encoding an antigenic polypeptide which is or is from *Serpulina hyodysenteriae*, Foot and Mouth Disease Virus, Hog Cholera Virus, African Swine Fever Virus or *Mycoplasma hyopneumoniae*.

The invention further provides for a recombinant swinepox virus capable of replication which contains foreign DNA encoding RNA encoding the antigenic polypeptide Newcastle Disease Virus (NDV) hemagglutinin-neuraminidase further comprising foreign DNA encoding a polypeptide which is a detectable marker.

The present invention further provides a recombinant swinepox virus which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox genome, wherein the foreign DNA sequence encodes an antigenic polypeptide derived from infectious bovine rhinotracheitis virus and is capable of being expressed in a host infected by the recombinant swinepox virus. Examples of such antigenic polypeptide are infectious bovine rhinotracheitis virus glycoprotein E and glycoprotein G.

The present invention further provides a recombinant swinepox virus which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox genome, wherein the foreign DNA sequence encodes an antigenic polypeptide derived from infectious laryngotracheitis virus and is capable of being expressed in a host infected by the recombinant swinepox virus. Examples of such antigenic polypeptide are infectious laryngotracheitis virus glycoprotein G and glycoprotein I.

In one embodiment of the recombinant swinepox virus the foreign DNA sequence encodes a cytokine. In another embodiment the cytokine is chicken myelomonocytic growth factor (cMGF) or chicken interferon (cIFN). Cytokines include, but are not limited to: transforming growth factor beta, epidermal growth factor family, fibroblast growth factors, hepatocyte growth factor, insulin-like growth factor, vascular endothelial growth factor,

interleukin 1, IL-1 receptor antagonist, interleukin-2, interleukin-3, interleukin-4, interleukin-5, interleukin-6, IL-6 soluble receptor, interleukin-7, interleukin-8, interleukin-9, interleukin-10, interleukin-11, interleukin-12, interleukin-13, angiogenin, chemokines, colony stimulating factors, granulocyte-macrophage colony stimulating factors, erythropoietin, interferon, interferon gamma, Stem cell factor (or known as mast cell growth factor, or c-kit ligand protein), leukemia inhibitory factor, oncostatin M, pleiotrophin, secretory leukocyte protease inhibitor, stem cell factor, tumor necrosis factors, and soluble TNF receptors. These cytokines are from humans, bovine, eqüine, feline, canine, porcine or avian.

The present invention further provides a recombinant swinepox virus which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox genome, wherein the foreign DNA sequence encodes an antigenic polypeptide derived from a human pathogen and is capable of being expressed in a host infected by the recombinant swinepox virus.

Recombinant SPV expressing cytokines is used to enhance the immune response either alone or when combined with vaccines containing cytokines or antigen genes of disease causing microorganisms.

Antigenic polypeptide of a human pathogen which are derived from human herpesvirus include, but are not limited to: hepatitis B virus and hepatitis C virus hepatitis B virus surface and core antigens, hepatitis C virus, human immunodeficiency virus, herpes simplex virus-1, herpes simplex virus-2, human cytomegalovirus, Epstein-Barr virus, Varicella-Zoster virus, human herpesvirus-6, human herpesvirus-7, human influenza, measles virus, hantaan virus, pneumonia virus,

rhinovirus, poliovirus, human respiratory syncytial virus, retrovirus, human T-cell leukemia virus, rabies virus, mumps virus, malaria (*Plasmodium falciparum*), *Bordetella pertussis*, Diptheria, *Rickettsia prowazekii*, *Borrelia berfdorferi*, Tetanus toxoid, malignant tumor antigens.

In one embodiment of the invention, a recombinant swinepox virus contains the foreign DNA sequence encoding hepatitis B virus core protein.

The present invention further provides a recombinant swinepox virus which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox genome, wherein the foreign DNA sequence encodes a cytokine capable of stimulating an immune in a host infected by the recombinant swinepox virus and is capable of being expressed in the host infected.

The present invention further provides a recombinant swinepox virus which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox genome, wherein the foreign DNA sequence encodes an antigenic polypeptide derived from an equine pathogen and is capable of being expressed in a host infected by the recombinant swinepox virus.

The antigenic polypeptide of an equine pathogen can derived from equine influenza virus, or equine herpesvirus. In one embodiment the antigenic polypeptide is equine influenza neuraminidase or hemagglutinin. Examples of such antigenic polypeptide are equine influenza virus type A/Alaska 91 neuraminidase, equine influenza virus type A/Prague 56 neuraminidase, equine influenza virus type A/Miami 63 neuraminidase, equine influenza virus type A/Kentucky 81 neuraminidase, equine

influenza virus type A/Kentucky 92 neuraminidase equine herpesvirus type 1 glycoprotein B, equine herpesvirus type 1 glycoprotein D, *Streptococcus equi*, equine infectious anemia virus, equine encephalitis virus, equine rhinovirus and equine rotavirus.

The present invention further provides an antigenic polypeptide which includes, but is not limited to: hog cholera virus gE1, hog cholera virus gE2, swine influenza virus hemagglutinin, neurominidase, matrix and nucleoprotein, pseudorabies virus gB, gC and gD, and PRRS virus ORF7.

The present invention further provides a recombinant swinepox virus which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox genome, wherein the foreign DNA sequence encodes an antigenic polypeptide derived from bovine respiratory syncytial virus or bovine parainfluenza virus, and is capable of being expressed in a host infected by the recombinant swinepox virus.

For example, the antigenic polypeptide of derived from infectious bovine rhinotracheitis virus gE, bovine respiratory syncytial virus equine pathogen can derived from equine influenza virus is bovine respiratory syncytial virus attachment protein (BRSV G), bovine respiratory syncytial virus fusion protein (BRSV F), bovine respiratory syncytial virus nucleocapsid protein (BRSV N), bovine parainfluenza virus type 3 fusion protein, and the bovine parainfluenza virus type 3 hemagglutinin neuraminidase.

The present invention further provides a recombinant swinepox virus which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox genome, wherein the foreign DNA sequence encodes bovine

viral diarrhea virus (BVDV) glycoprotein 48 or glycoprotein 53, and wherein the foreign DNA sequence is capable of being expressed in a host infected by the recombinant swinepox virus.

The present invention further provides a recombinant swinepox virus which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox genome, wherein the foreign DNA sequence encodes an antigenic polypeptide derived from infectious bursal disease virus and wherein the foreign DNA sequence is capable of being expressed in a host infected by the recombinant swinepox virus. Examples of such antigenic polypeptide are infectious bursal disease virus polyprotein and VP2.

The present invention further provides a recombinant swinepox virus in which the foreign DNA sequence encodes an antigenic polypeptide which includes, but is not limited to: MDV gA, MDV gB, MDV gD, NDV HN, NDV F, ILT gB, ILT gI, ILT gD, IBDV VP2, IBDV VP3, IBDV VP4, IBDV polyprotein, IBV spike, IBV matrix, avian encephalomyelitis virus, avian reovirus, avian paramyxovirus, avian influenza virus, avian adenovirus, fowl pox virus, avian coronavirus, avian rotavirus, chick anemia virus, *Salmonella spp. E. coli, Pasteurella spp., Bordetella spp., Eimeria spp., Histomonas spp., Trichomonas spp.*, Poultry nematodes, cestodes, trematodes, poultry mites/lice, and poultry protozoa.

The invention further provides that the inserted foreign DNA sequence is under the control of a promoter. In one embodiment the is a swinepox viral promoter. In another embodiment the foreign DNA sequence is under control of an endogenous upstream poxvirus promoter. In another embodiment the foreign DNA sequence is under control of a heterologous upstream promoter.

For purposes of this invention, promoters include but is not limited to: synthetic pox viral promoter, pox synthetic late promoter 1, pox synthetic late promoter 2 early promoter 2, pox O1L promoter, pox I4L promoter, pox I3L promoter, pox I2L promoter, pox I1L promoter, pox E10R promoter, PRV gX, HSV-1 alpha 4, HCMV immediate early, MDV gA, MDV gB, MDV gD, ILT gB, BHV-1.1 VP8 and ILT gD and internal ribosomal entry site promoter. Alternate promoters are generated by methods well known to those of skill in the art, for example, as set forth in the STRATEGY FOR THE CONSTRUCTION OF SYNTHETIC POX VIRAL PROMOTERS in Materials and Methods.

The invention provides for a homology vector for producing a recombinant swinepox virus by inserting foreign DNA into the genomic DNA of a swinepox virus. The homology vector comprises a double-stranded DNA molecule consisting essentially of a double-stranded foreign DNA sequence or (RNA) which does not naturally occur in an animal into which the recombinant swinepox virus is introduced, with at one end of the foreign DNA, double-stranded swinepox viral DNA homologous to genomic DNA located at one side of a site on the genomic DNA which is not essential for replication of the swinepox virus, and at the other end of the foreign DNA, double-stranded swinepox viral DNA homologous to genomic DNA located at the other side of the same site on the genomic DNA. Preferably, the RNA encodes a polypeptide.

In another embodiment of the present invention, the double-stranded swinepox viral DNA of the homology vectors described above is homologous to genomic DNA present within the HindIII M fragment. In another embodiment the double-stranded swinepox viral DNA of the homology vectors described above is homologous to genomic DNA present within an approximately 2 Kb HindIII to BglII sub-fragment. In a preferred embodiment the double-

stranded swinepox viral DNA is homologous to genomic DNA present within the *Bgl*II site located in this *Hind*III to *Bgl*II subfragment.

In another embodiment the double-stranded swinepox viral DNA is homologous to genomic DNA present within the open reading frame contained in the larger *Hind*III to *Bgl*II subfragment. Preferably, the double-stranded swinepox viral DNA is homologous to genomic DNA present within the *Acc*I restriction endonuclease site located in the larger *Hind*III to *Bgl*II subfragment.

In one embodiment, the polypeptide is a detectable marker. Preferably, the polypeptide which is a detectable marker is *E. coli* ß-galactosidase.

In one embodiment, the foreign DNA which codes for a polypeptide include but are not limited to: feline leukemia virus surface protein, feline leukemia virus transmembrane protein, feline leukemia virus gag, feline leukemia virus transmembrane protease, feline immunodeficiency virus gag/protease, feline immunodeficiency virus envelope, feline leukemia virus gag/protease, feline leukemia virus envelope, canine parvovirus VP2, canine parvovirus VP1/2, bovine cytokine interleukin-12 protein 35, bovine cytokine interleukin-12 protein 40, Bovine Respiratory Syncytial Virus glycoprotein G, Newcastles Disease fusion, Infectious Rhinotracheitis Virus glycoprotein D, Canine Distemper Virus fusion, Canine Distemper Virus Hemagluttinin, DV HA, Bovine Viral Diarrhea Virus type 1 glycoprotein 45, Bovine Viral Diarrhea Virus type 1 glycoprotein 48, Bovine Viral Diarrhea Virus type 1 glycoprotein 53, Bovine Viral Diarrhea Virus type 2 glycoprotein 53.

Other polypeptides include: pseudorabies virus (PRV) g50 (gD), pseudorabies virus (PRV) gII (gB), Pseudorabies virus (PRV) gIII (gC), Pseudorabies virus (PRV) glycoprotein H, Transmissible gastroenteritis (TGE) glycoprotein 195, Transmissible gastroenteritis (TGE) matrix protein, swine rotavirus glycoprotein 38, swine parvovirus capsid protein, *Serpulina hydodysenteriae* protective antigen, Bovine Viral Diarrhea (BVD) glycoprotein 53 and g48, Newcastle Disease Virus (NDV) hemagglutinin-neuraminidase, swine flu hemagglutinin or swine flu neuraminidase. Preferably, the antigenic polypeptide is or is from *Serpulina hyodysenteriae*, Foot and Mouth Disease Virus, Hog Cholera Virus gE1 and gE2, Swine Influenza Virus, African Swine Fever Virus or *Mycoplasma hyopneumoniae*, swine influenza virus hemagglutinin, neuraminidase and matrix and nucleoprotein, PRRS virus ORF7, and hepatitis B virus core protein.

In one embodiment, the polypeptide is antigenic in the animal.

In an embodiment of the present invention, the double stranded foreign DNA sequence in the homology vector encodes an antigenic polypeptide derived from a human pathogen.

For example, the antigenic polypeptide of a human pathogen is derived from human herpesvirus, herpes simplex virus-1, herpes simplex virus-2, human cytomegalovirus, Epstein-Barr virus, Varicell-Zoster virus, human herpesvirus-6, human herpesvirus-7, human influenza, human immunodeficiency virus, rabies virus, measles virus, hepatitis B virus and hepatitis C virus. Furthermore, the antigenic polypeptide of a human pathogen may be associated with malaria or malignant

tumor from the group conisting of *Plasmodium falciparum*, Bordetella pertusis, and malignant tumor.

In an embodiment of the present invention, the double stranded foreign DNA sequence in the homology vector encodes a cytokine capable of stimulating human immune response. In one embodiment the cytokine is a chicken myelomonocytic growth factor (cMGF) or chicken interferon (cIFN). For example, the cytokine can be, but not limited to, interleukin-2, interleukin-6, interleukin-12, interferons, granulocyte-macrophage colony stimulating factors, and interleukin receptors.

In an embodiment of the present invention, the double stranded foreign DNA sequence in the homology vector encodes an antigenic polypeptide derived from an equine pathogen.

The antigenic polypeptide of an equine pathogen can derived from equine influenza virus or equine herpesvirus. Examples of such antigenic polypeptide are equine influenza virus type A/Alaska 91 neuraminidase, equine influenza virus type A/Prague 56 neuraminidase, equine influenza virus type A/Miami 63 neuraminidase, equine influenza virus type A/Kentucky 81 neuraminidase equine herpesvirus type 1 glycoprotein B, and equine herpesvirus type 1 glycoprotein D.

In an embodiment of the present invention, the double stranded foreign DNA sequence of the homology vector encodes an antigenic polypeptide derived from bovine respiratory syncytial virus or bovine parainfluenza virus.

For example, the antigenic polypeptide is derived from infectious bovine rhinotracheitis gE, bovine respiratory syncytial virus attachment protein (BRSV G), bovine

respiratory syncytial virus fusion protein (BRSV F), bovine respiratory syncytial virus nucleocapsid protein (BRSV N), bovine parainfluenza virus type 3 fusion protein, and the bovine parainfluenza virus type 3 hemagglutinin neuraminidase.

In an embodiment of the present invention, the double stranded foreign DNA sequence of the homology vector encodes an antigenic polypeptide derived from infectious bursal disease virus. Examples of such antigenic polypeptide are infectious bursal disease virus polyprotein and infectious bursal disease virus VP2, VP3, or VP4.

For purposes of this invention, a "homology vector" is a plasmid constructed to insert foreign DNA in a specific site on the genome of a swinepox virus.

In one embodiment of the invention, the double-stranded swinepox viral DNA of the homology vectors described above is homologous to genomic DNA present within the open reading frame encoding swinepox thymidine kinase. Preferably, the double-stranded swinepox viral DNA is homologous to genomic DNA present within the *Nde*I restriction endonuclease site located in the open reading frame encoding swinepox thymidine kinase.

The invention further provides a homology vectors described above, the foreign DNA sequence of which is under control of a promoter located upstream of the foreign DNA sequence. The promoter can be an endogenous swinepox viral promoter or an exogenous promoter. Promoters include, but are not limited to: synthetic pox viral promoter, pox synthetic late promoter 1, pox synthetic late promoter 2 early promoter 2, pox O1L promoter, pox I4L promoter, pox I3L promoter, pox I2L promoter, pox I1L promoter, pox E10R promoter, PRV gX,

HSV-1 alpha 4, HCMV immediate early, BHV-1.1 VP8, infectious laryngotracheitis virus glycoprotein B, infectious laryngotracheitis virus gD, marek's disease virus glycoprotein A, marek's disease virus glycoprotein B, and marek's disease virus glycoprotein D.

The invention further provides a vaccine which comprises an effective immunizing amount of a recombinant swinepox virus of the present invention and a suitable carrier.

Suitable carriers for the swinepox virus are well known in the art and include proteins, sugars, etc. One example of such a suitable carrier is a physiologically balanced culture medium containing one or more stabilizing agents such as stabilized, hydrolyzed proteins, lactose, etc.

For purposes of this invention, an "effective immunizing amount" of the recombinant swinepox virus of the present invention is within the range of 10^3 to 10^9 PFU/dose.

The present invention also provides a method of immunizing an animal, wherein the animal is a human, swine, bovine, equine, caprine or ovine. For purposes of this invention, this includes immunizing the animal against the virus or viruses which cause the disease or diseases pseudorabies, transmissible gastroenteritis, swine rotavirus, swine parvovirus, *Serpulina hyodysenteriae*, bovine viral diarrhea, Newcastle disease, swine influenza, PRRS, bovine respiratory synctial virus, bovine parainfluenza virus type 3, foot and mouth disease, hog cholera, African swine fever or *Mycoplasma hyopneumoniae*. For purposes of this invention, the method of immunizing also includes immunizing the animal against human pathogens, bovine pathogens, equine

pathogens, avian pathogens described in the preceding part of this section.

The method comprises administering to the animal an effective immunizing dose of the vaccine of the present invention. The vaccine may be administered by any of the methods well known to those skilled in the art, for example, by intramuscular, subcutaneous, intraperitoneal or intravenous injection. Alternatively, the vaccine may be administered intranasally or orally.

The present invention also provides a method for testing a swine to determine whether the swine has been vaccinated with the vaccine of the present invention, particularly the embodiment which contains the recombinant swinepox virus S-SPV-008 (ATCC Accession No. VR 2339), or is infected with a naturally-occurring, wild-type pseudorabies virus. This method comprises obtaining from the swine to be tested a sample of a suitable body fluid, detecting in the sample the presence of antibodies to pseudorabies virus, the absence of such antibodies indicating that the swine has been neither vaccinated nor infected, and for the swine in which antibodies to pseudorabies virus are present, detecting in the sample the absence of antibodies to pseudorabies virus antigens which are normally present in the body fluid of a swine infected by the naturally-occurring pseudorabies virus but which are not present in a vaccinated swine indicating that the swine was vaccinated and is not infected.

The present invention provides a recombinant SPV which when inserted with a foreign DNA sequence or gene may be employed as a diagnostic assay. In one embodiment FIV *env* and *gag* genes and *D. immitis* p39 and 22kd are employed in a diagnostic assay to detect feline

immunodeficiency caused by FIV and to detect heartworm caused by *D. immits*, respectively.

The present invention also provides a host cell infected with a recombinant swinepox virus capable of replication. In one embodiment, the host cell is a mammalian cell. Preferably, the mammalian cell is a Vero cell. Preferably, the mammalian cell is an ESK-4 cell, PK-15 cell or EMSK cell.

For purposes of this invention a "host cell" is a cell used to propagate a vector and its insert. Infecting the cells was accomplished by methods well known to those of skill in the art, for example, as set forth in INFECTION - TRANSFECTION PROCEDURE in Material and Methods.

Methods for constructing, selecting and purifying recombinant swinepox viruses described above are detailed below in Materials and Methods.

EXPERIMENTAL DETAILS

Materials and Methods

PREPARATION OF SWINEPOX VIRUS STOCK SAMPLES. Swinepox virus (SPV) samples were prepared by infecting embryonic swine kidney (EMSK) cells, ESK-4 cells, PK-15 cells or Vero cells at a multiplicity of infection of 0.01 PFU/cell in a 1:1 mixture of Iscove's Modified Dulbecco's Medium (IMDM) and RPMI 1640 medium containing 2 mM glutamine, 100 units/ml penicillin, 100 units/ml streptomycin (these components were obtained from Sigma or equivalent supplier, and hereafter are referred to as EMSK negative medium). Prior to infection, the cell monolayers were washed once with EMSK negative medium to remove traces of fetal bovine serum. The SPV contained in the initial inoculum (0.5 ml for 10 cm plate; 10 ml for T175 cm flask) was then allowed to absorb onto the cell monolayer for two hours, being redistributed every half hour. After this period, the original inoculum was brought up to the recommended volume with the addition of complete EMSK medium (EMSK negative medium plus 5% fetal bovine serum). The plates were incubated at 37°C in 5% CO_2 until cytopathic effect was complete. The medium and cells were harvested and frozen in a 50 ml conical screw cap tube at -70°C. Upon thawing at 37°C, the virus stock was aliquoted into 1.0 ml vials and refrozen at -70°C. The titers were usually about 10^6 PFU/ml.

PREPARATION OF SPV DNA. For swinepox virus DNA isolation, a confluent monolayer of EMSK cells in a T175 cm^2 flask was infected at a multiplicity of 0.1 and incubated 4-6 days until the cells were showing 100% cytopathic effect. The infected cells were then harvested by scraping the cells into the medium and centrifuging at 3000 rpm for 5 minutes in a clinical centrifuge. The medium was decanted, and the cell pellet was gently resuspended in

1.0 ml Phosphate Buffer Saline (PBS: 1.5g Na_2HPO_4, 0.2g KH_2PO_4, 0.8g NaCL and 0.2g KCl per liter H_2O) (per T175) and subjected to two successive freeze-thaws (-70° C to 37° C). Upon the last thaw, the cells (on ice) were sonicated two times for 30 seconds each with 45 seconds cooling time in between. Cellular debris was then removed by centrifuging (Sorvall RC-5B superspeed centrifuge) at 3000 rpm for 5 minutes in a HB4 rotor at 4° C. SPV virions, present in the supernatant, were then pelleted by centrifugation at 15,000 rpm for 20 minutes at 4° C in a SS34 rotor (Sorvall) and resuspended in 10 mM Tris (pH 7.5). This fraction was then layered onto a 36% sucrose gradient (w/v in 10 mM tris pH 7.5) and centrifuged (Beckman L8-70M Ultracentrifuge) at 18,000 rpm for 60 minutes in a SW41 rotor (Beckman) at 4° C. The virion pellet was resuspended in 1.0 ml of 10 mM tris pH 7.5 and sonicated on ice for 30 seconds. This fraction was layered onto a 20% to 50% continuous sucrose gradient and centrifuged 16,000 rpm for 60 minutes in a SW41 rotor at 4° C. The SPV virion band located about three quarters down the gradient was harvested, diluted with 20% sucrose and pelleted by centrifugation at 18,000 rpm for 60 minutes in a SW41 rotor at 4° C. The resultant pellet was then washed once with 10 mM Tris pH 7.5 to remove traces of sucrose and finally resuspended in 10 mM Tris pH 7.5. SPV DNA was then extracted from the purified virions by lysis (4 hours at 60° C) induced by the addition of EDTA, SDS, and proteinase K to final concentrations of 20 mM, 0.5% and 0.5 mg/ml, respectively. After digestion, three phenol:chloroform (1:1) extractions were conducted and the sample precipitated by the addition of two volumes of absolute ethanol and incubation at -20° C for 30 minutes. The sample was then centrifuged in an Eppendorf minifuge for 5 minutes at full speed. The supernatant was decanted, and the pellet air dried and rehydrated in 0.01 M Tris pH 7.5, 1 mM EDTA at 4° C.

PREPARATION OF INFECTED CELL LYSATES. For cell lysate preparation, serum free medium was used. A confluent monolayer of cells (EMSK, ESK-4, PK-15 or Vero for SPV or VERO for PRV) in a 25 cm^2 flask or a 60 mm petri dish was infected with 100 μl of virus sample. After cytopathic effect was complete, the medium and cells were harvested and the cells were pelleted at 3000 rpm for 5 minutes in a clinical centrifuge. The cell pellet was resuspended in 250 μl of disruption buffer (2% sodium dodecyl sulfate, 2% ß-mercapto-ethanol). The samples were sonicated for 30 seconds on ice and stored at -20°C.

WESTERN BLOTTING PROCEDURE. Samples of lysates and protein standards were run on a polyacrylamide gel according to the procedure of Laemnli (1970). After gel electrophoresis the proteins were transferred and processed according to Sambrook *et al.* (1982). The primary antibody was a swine anti-PRV serum (Shope strain; lot370, PDV8201, NVSL, Ames, IA) diluted 1:100 with 5% non-fat dry milk in Tris-sodium chloride, and sodium Azide (TSA: 6.61g Tris-HCl, 0.97g Tris-base, 9.0g NaCl and 2.0g Sodium Azide per liter H_2O). The secondary antibody was a goat anti-swine alkaline phosphatase conjugate diluted 1:1000 with TSA.

MOLECULAR BIOLOGICAL TECHNIQUES. Techniques for the manipulation of bacteria and DNA, including such procedures as digestion with restriction endonucleases, gel electrophoresis, extraction of DNA from gels, ligation, phosphorylation with kinase, treatment with phosphatase, growth of bacterial cultures, transformation of bacteria with DNA, and other molecular biological methods are described by Maniatis *et al.* (1982) and Sambrook *et al.* (1989). Except as noted, these were used with minor variation.

DNA SEQUENCING. Sequencing was performed using the USB Sequenase Kit and ^{35}S-dATP (NEN). Reactions using both the dGTP mixes and the dITP mixes were performed to clarify areas of compression. Alternatively, compressed areas were resolved on formamide gels. Templates were double-stranded plasmid subclones or single stranded M13 subclones, and primers were either made to the vector just outside the insert to be sequenced, or to previously obtained sequence. Sequence obtained was assembled and compared using Dnastar software. Manipulation and comparison of sequences obtained was performed with Superclone™ and Supersee™ programs from Coral Software.

CLONING WITH THE POLYMERASE CHAIN REACTION. The polymerase chain reaction (PCR) was used to introduce restriction sites convenient for the manipulation of various DNAs. The procedures used are described by Innis, *et al*. (1990). In general, amplified fragments were less than 500 base pairs in size and critical regions of amplified fragments were confirmed by DNA sequencing. The primers used in each case are detailed in the descriptions of the construction of homology vectors below.

HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. This method relies upon the homologous recombination between the swinepox virus DNA and the plasmid homology vector DNA which occurs in the tissue culture cells containing both swinepox virus DNA and transfected plasmid homology vector. For homologous recombination to occur, the monolayers of EMSK cells are infected with S-SPV-001 (Kasza SPV strain, 17) at a multiplicity of infection of 0.01 PFU/cell to introduce replicating SPV (i.e. DNA synthesis) into the cells. The plasmid homology vector DNA is then transfected into these cells according to the INFECTION - TRANSFECTION

PROCEDURE. The construction of homology vectors used in this procedure is described below

INFECTION - TRANSFECTION PROCEDURE. 6 cm plates of EMSK cells (about 80% confluent) were infected with S-SPV-001 at a multiplicity of infection of 0.01 PFU/cell in EMSK negative medium and incubated at 37°C in a humidified 5% CO_2 environment for 5 hours. The transfection procedure used is essentially that recommended for Lipofectin™ Reagent (BRL). Briefly, for each 6 cm plate, 15 μg of plasmid DNA was diluted up to 100 μl with H_2O. Separately, 50 micrograms of Lipofectin Reagent was diluted to 100 μl with H_2O. The 100 μl of diluted Lipofectin Reagent was then added dropwise to the diluted plasmid DNA contained in a polystyrene 5 ml snap cap tube and mixed gently. The mixture was then incubated for 15-20 minutes at room temperature. During this time, the virus inoculum was removed from the 6 cm plates and the cell monolayers washed once with EMSK negative medium. Three ml of EMSK negative medium was then added to the plasmid DNA/lipofectin mixture and the contents pipetted onto the cell monolayer. The cells were incubated overnight (about 16 hours) at 37°C in a humidified 5% CO_2 environment. The next day the 3 ml of EMSK negative medium was removed and replaced with 5 ml EMSK complete medium. The cells were incubated at 37°C in 5% CO_2 for 3-7 days until cytopathic effect from the virus was 80-100%. Virus was harvested as described above for the preparation of virus stocks. This stock was referred to as a transfection stock and was subsequently screened for recombinant virus by the BLUOGAL SCREEN FOR RECOMBINANT SWINEPOX VIRUS OR CPRG SCREEN FOR RECOMBINANT SWINEPOX VIRUS.

SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). When the *E. coli* ß-galactosidase (lacZ) marker gene was incorporated into a

recombinant virus the plaques containing the recombinants were visualized by one of two simple methods. In the first method, the chemical Bluogal™ (Bethesda Research Labs) was incorporated (200 μg/ml) into the agarose overlay during the plaque assay, and plaques expressing active ß-galactosidase turned blue. The blue plaques were then picked onto fresh cells (EMSK) and purified by further blue plaque isolation. In the second method, CPRG (Boehringer Mannheim) was incorporated (400 μg/ml) into the agarose overlay during the plaque assay, and plaques expressing active ß-galactosidase turned red. The red plaques were then picked onto fresh cells (EMSK) and purified by further red plaque isolation. In both cases viruses were typically purified with three rounds of plaque purification.

SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV USING BLACK PLAQUE ASSAYS. To analyze expression of foreign antigens expressed by recombinant swinepox viruses, monolayers of EMSK cells were infected with recombinant SPV, overlayed with nutrient agarose media and incubated for 6-7 days at 37°C for plaque development to occur. The agarose overlay was then removed from the dish, the cells fixed with 100% methanol for 10 minutes at room temperature and the cells air dried. Fixation of the cells results in cytoplasmic antigen as well as surface antigen detection whereas specific surface antigen expression can be detected using non-fixed cells. The primary antibody was then diluted to the appropriate dilution with PBS and incubated on the cell monolayer for 2 hours at room temperature. To detect PRV g50 (gD) expression from S-SPV-008, swine anti-PRV serum (Shope strain; lot370, PDV8201, NVSL, Ames, IA) was used (diluted 1:100). To detect NDV HN expression from S-SPV-009, a rabbit antiserum specific for the HN protein (rabbit anti-NDV#2) was used (diluted 1:1000). Unbound antibody was then removed by washing the cells three

times with PBS at room temperature. The secondary antibody, either a goat anti-swine (PRV g50 (gD); S-SPV-008) or goat anti-rabbit (NDV HN; S-SPV-009), horseradish peroxidase conjugate was diluted 1:250 with PBS and incubated with the cells for 2 hours at room temperature. Unbound secondary antibody was then removed by washing the cells three times with PBS at room temperature. The cells were then incubated 15-30 minutes at room temperature with freshly prepared substrate solution (100 μg/ml 4-chloro-1-naphthol, 0.003% H_2O_2 in PBS). Plaques expressing the correct antigen stain black.

PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS. Viral glycoproteins are purified using antibody affinity columns. To produce monoclonal antibodies, 8 to 10 week old BALB/c female mice are vaccinated intraperitoneally seven times at two to four week intervals with 10^7 PFU of S-SPV-009, -014, -016, -017, -018, or -019. Three weeks after the last vaccination, mice are injected intraperitoneally with 40 mg of the corresponding viral glycoprotein. Spleens are removed from the mice three days after the last antigen dose.

Splenocytes are fused with mouse NS1/Ag4 plasmacytoma cells by the procedure modified from Oi and Herzenberg, (41). Splenocytes and plasmacytoma cells are pelleted together by centrifugation at 300 x g for 10 minutes. One ml of a 50% solution of polyethylene glycol (m.w. 1300-1600) is added to the cell pellet with stirring over one minute. Dulbecco's modified Eagles's medium (5ml) is added to the cells over three minutes. Cells are pelleted by centrifugation at 300 x g for 10 minutes and resuspended in medium with 10% fetal bovine serum and containing 100 mM hypoxanthine, 0.4 mM aminopterin and 16 mM thymidine (HAT). Cells (100 ml) are added to the wells of eight to ten 96-well tissue culture plates

containing 100 ml of normal spleen feeder layer cells and incubated at 37°C. Cells are fed with fresh HAT medium every three to four days.

Hybridoma culture supernatants are tested by the ELISA ASSAY in 96-well microtiter plates coated with 100 ng of viral glycoprotein. Supernatants from reactive hybridomas are further analyzed by black-plaque assay and by Western Blot. Selected hybridomas are cloned twice by limiting dilution. Ascetic fluid is produced by intraperitoneal injection of 5 x 10^6 hybridoma cells into pristane-treated BALB/c mice.

Cell lysates from S-SPV-009, -014, -016, -017, -018, or -019 are obtained as described in PREPARATION OF INFECTED CELL LYSATES. The glycoprotein-containing cell lysates (100 mls) are passed through a 2-ml agarose affinity resin to which 20 mg of glycoprotein monoclonal antibody has been immobilized according to manufacturer's instructions (AFC Medium, New Brunswick Scientific, Edison, N.J.). The column is washed with 100 ml of 0.1% Nonidet P-40 in phosphate-buffered saline (PBS) to remove nonspecifically bound material. Bound glycoprotein is eluted with 100 mM carbonate buffer, pH 10.6 (40). Pre- and posteluted fractions are monitored for purity by reactivity to the SPV monoclonal antibodies in an ELISA system.

ELISA ASSAY. A standard enzyme-linked immunosorbent assay (ELISA) protocol is used to determine the immune status of cattle following vaccination and challenge.

A glycoprotein antigen solution (100 ml at ng/ml in PBS) is allowed to absorb to the wells of microtiter dishes for 18 hours at 4°C. The coated wells are rinsed one time with PBS. Wells are blocked by adding 250 ml of PBS containing 1% BSA (Sigma) and incubating 1 hour at 37°C.

The blocked wells are rinsed one time with PBS containing 0.02% Tween 20. 50 ml of test serum (previously diluted 1:2 in PBS containing 1% BSA) are added to the wells and incubated 1 hour at 37°C. The antiserum is removed and the wells are washed 3 times with PBS containing 0.02% Tween 20. 50 ml of a solution containing anti-bovine IgG coupled to horseradish peroxidase (diluted 1:500 in PBS containing 1% BSA, Kirkegaard and Perry Laboratories, Inc.) is added to visualize the wells containing antibody against the specific antigen. The solution is incubated 1 hour at 37°C, then removed and the wells are washed 3 times with PBS containing 0.02% Tween 20. 100 ml of substrate solution (ATBS, Kirkegaard and Perry Laboratories, Inc.) are added to each well and color is allowed to develop for 15 minutes. The reaction is terminated by addition of 0.1M oxalic acid. The color is read at absorbance 410nm on an automatic plate reader.

STRATEGY FOR THE CONSTRUCTION OF SYNTHETIC POX VIRAL PROMOTERS. For recombinant swinepox vectors synthetic pox promoters offer several advantages including the ability to control the strength and timing of foreign gene expression. Three promoter cassettes LP1, EP1 and LP2 based on promoters that have been defined in the vaccinia virus (1, 7 and 8) were designed. Each cassette was designed to contain the DNA sequences defined in vaccinia flanked by restriction sites which could be used to combine the cassettes in any order or combination. Initiator methionines were also designed into each cassette such that inframe fusions could be made at either *Eco*RI or *Bam*HI sites. A set of translational stop codons in all three reading frames and an early transcriptional termination signal (9) were also engineered downstream of the inframe fusion site. DNA encoding each cassette was synthesized according to standard techniques and cloned into the appropriate homology vectors (see Figures 3 and 4).

VACCINATION STUDIES IN SWINE USING RECOMBINANT SWINEPOX VIRUS CONTAINING PSEUDORABIES VIRUS GLYCOPROTEIN GENES: Young weaned pigs from pseudorabies-free herd are used to test the efficacy of the recombinant swinepox virus containing one or more of the pseudorabies virus glycoprotein genes (SPV/PRV). The piglets are inoculated intramuscularly, intradermally or orally about 10^3 to 10^7 plaque forming units (PFU) of the recombinant SPV/PRV viruses.

Immunity is determined by measuring PRV serum antibody levels and by challenging the vaccinated pigs with virulent strain of pseudorabies virus. Three to four weeks post-vaccination, both vaccinated and non-vaccinated groups of pigs are challenged with virulent strain of pseudorabies virus (VDL4892). Post challenge, the pigs are observed daily for 14 days for clinical signs of pseudorabies.

Serum samples are obtained at the time of vaccination, challenge, and at weekly intervals for two to three weeks post-vaccination and assayed for serum neutralizing antibody.

CLONING OF BOVINE VIRAL DIARRHEA VIRUS g48 and g53 GENES. The bovine viral diarrhea g48 and g53 genes were cloned by a PCR CLONING procedure essentially as described by Katz *et al.* (42) for the HA gene of human influenza. Viral RNA prepared from BVD virus Singer strain grown in Madin-Darby bovine kidney (MDBK) cells was first converted to cDNA utilizing an oligonucleotide primer specific for the target gene. The cDNA was then used as a template for polymerase chain reaction (PCR) cloning (15) of the targeted region. The PCR primers were designed to incorporate restriction sites which permit the cloning of the amplified coding regions into vectors

containing the appropriate signals for expression in SPV. One pair of oligonucleotides were required for each coding region. The g48 gene coding region from the BVDV Singer strain (49) was cloned using the following primers: 5'-ACGTCGGATCCCTTACCAAACCACGTCTTACTCTTGTTTTCC-3' for cDNA priming and combined with 5'-ACATAGGATCCCATGGGAGAAAACATAACACAGTGGAACC-3' for PCR. The g53 gene coding region from the BVDV Singer strain (49) was cloned using the following primers: 5'-CGTGGATCCTCAATTACAAGAGGTATCGTCTAC-3' for cDNA priming and combined with 5'-CATAGATCTTGTGGTGCTGTCCGACTTCGCA-3' for PCR. Note that this general strategy is used to clone the coding region of g48 and g53 genes from other strains of BVDV. The DNA fragment for BVDV g 48 was digested with *Bam*HI to yield an 678 bp fragment. The DNA fragment for BVDV g53 was digested with *Bgl*II and *Bam*HI to yield an 1187 bp fragment. The BVDV g48 or g53 DNA fragments were cloned into the *Bam*HI site next to the LP2EP2 promoter of the SPV homology vector to yield homology vectors, 727-78.1 and 738-96, respectively.

CLONING OF BOVINE RESPIRATORY SYNCYTIAL VIRUS FUSION, NUCLEOCAPSID AND GLYCOPROTEIN GENES. The bovine respiratory syncytial virus fusion (F), nucleocapsid (N), and glycoprotein (G) genes were cloned by a PCR CLONING procedure essentially as described by Katz *et al.* (42) for the HA gene of human influenza. Viral RNA prepared from BRSV virus grown in bovine nasal turbinate (BT) cells was first converted to cDNA utilizing an oligonucleotide primer specific for the target gene. The cDNA was then used as a template for polymerase chain reaction (PCR) cloning (15) of the targeted region. The PCR primers were designed to incorporate restriction sites which permit the cloning of the amplified coding regions into vectors containing the appropriate signals for expression in SPV. One pair of oligonucleotides were required for each coding region. The F gene coding region

from the BRSV strain 375 (VR-1339) was cloned using the following primers: 5'-TGCAGGATCCTCATTTACTAAAGGAAAGATTGTTGAT-3' for cDNA priming and combined with 5'-CTCTGGATCCTACAGCCATGAGGATGATCATCAGC-3' for PCR. The N gene coding region from BRSV strain 375 (VR-1339) was cloned utilizing the following primers: 5'-CGTCGGATCCCTCACAGTTCCACATCATTGTCTTTGGGAT-3' for cDNA priming and combined with 5'-CTTAGGATCCCATGGCTCTTAGCAAGGTCAAACTAAATGAC-3' for PCR. The G gene coding region from BRSV strain 375 (VR-1339) was cloned utilizing the following primers: 5'-CGTTGGATCCCTAGATCTGTGTAGTTGATTGATTTGTGTGA-3' for cDNA priming and combined with 5'-CTCTGGATCCTCATACCCATCATCTTAAATTCAAGACATTA-3' for PCR. Note that this general strategy is used to clone the coding region of F, N and G genes from other strains of BRSV. The DNA fragments for BRSV F, N, or G were digested with *Bam*HI to yield 1722 bp, 1173 bp, or 771 bp fragments, respectively. The BRSV F, N, and G DNA fragments were cloned into the *Bam*HI site next to the LP2EP2 promoter of the SPV homology vector to yield homology vectors, 727-20.10, 713-55.37 and 727-20.5, respectively.

RNA ISOLATED FROM CONCANAVALIN A STIMULATED CHICKEN SPLEEN CELLS. Chicken spleens were dissected from 3 week old SPAFAS hatched chicks, washed, and disrupted through a syringe/needle to release cells. After allowing stroma and debri to settle out, the cells were pelleted and washed twice with PBS. The cell pellet was treated with a hypotonic lysis buffer to lyse red blood cells, and splenocytes were recovered and washed twice with PBS. Splenocytes were resuspended at 5 x 10^6 cells/ml in RPMI containing 5% FBS and 5 μg/ml Concanavalin A and incubated at 39° for 48 hours. Total RNA was isolated from the cells using guanidine isothionate lysis reagents

and protocols from the Promega RNA isolation kit (Promega Corporation, Madison WI). 4μg of total RNA was used in each 1st strand reaction containing the appropriate antisense primers and AMV reverse transcriptase (Promega Corporation, Madison WI). cDNA synthesis was performed in the same tube following the reverse transcriptase reaction, using the appropriate sense primers and Vent® DNA polymerase (Life Technologies, Inc. Bethesda, MD).

SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES. When the E.coli ß-glucuronidase (uidA) marker gene was incorporated into a recombinant virus the plaques containing recombinants were visualized by a simple assay. The enzymatic substrate was incorporated (300 μg/ml) into the agarose overlay during the plaque assay. For the uidA marker gene the substrate X-Glucuro Chx (5-bromo-4-chloro-3-indolyl-ß-D-glucuronic acid Cyclohexylammonium salt, Biosynth AG) was used. Plaques that expressed active marker enzyme turned blue. The blue plaques were then picked onto fresh ESK-4 cells and purified by further blue plaque isolation. In recombinant virus strategies in which the enzymatic marker gene is removed the assay involves plaque purifying white plaques from a background of parental blue plaques. In both cases viruses were typically purified with three rounds of plaque purification.

HOMOLOGY VECTOR 515-85.1. The plasmid 515-85.1 was constructed for the purpose of inserting foreign DNA into SPV. It contains a unique *Acc*I restriction enzyme site into which foreign DNA may be inserted. When a plasmid, containing a foreign DNA insert at the *Acc*I site, is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing the foreign DNA will result. A restriction map of the DNA insert in homology vector 515-85.1 is given in Figures 3A-3C. It may be constructed utilizing standard recombinant DNA

techniques (22 and 29), by joining two restriction fragments from the following sources. The first fragment is an approximately 2972 base pair *Hind*III to *Bam*HI restriction fragment of pSP64 (Promega). The second fragment is an approximately 3628 base pair *Hind*III to *Bgl*II restriction sub-fragment of the SPV *Hind*III restriction fragment M (23).

HOMOLOGY VECTOR 520-17.5. The plasmid 520-17.5 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an *E. coli* ß-galactosidase (lacZ) marker gene flanked by SPV DNA. Upstream of the marker gene is an approximately 2149 base pair fragment of SPV DNA. Downstream of the marker gene is an approximately 1484 base pair fragment of SPV DNA. When this plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the marker gene will result. Note that the ß-galactosidase (lacZ) marker gene is under the control of a synthetic early/late pox promoter. A detailed description of the plasmid is given in Figures 3A-3C. It may be constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the synthetic DNA sequences indicated in Figures 3A-3C. The plasmid vector was derived from an approximately 2972 base pair *Hind*III to *Bam*HI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 2149 base pair *Hind*III to *Acc*I restriction sub-fragment of the SPV *Hind*III restriction fragment M (23). Fragment 2 is an approximately 3006 base pair *Bam*HI to *Pvu*II restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 1484 base pair *Acc*I to *Bgl*II restriction sub-fragment of the SPV *Hind*III fragment M (23).

HOMOLOGY VECTOR 538-46.16. The plasmid 538-46.16 was constructed for the purpose of inserting foreign DNA into

SPV. It incorporates an *E. coli* ß-galactosidase (lacZ) marker gene and the PRV g50 (gD) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. When this plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes will result. Note that the ß-galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1) and the g50 (gD) gene is under the control of a synthetic early/late pox promoter (EP1LP2). A detailed description of the plasmid is given in 3A-3C. It may be constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the synthetic DNA sequences indicated in Figures 3A-3C. The plasmid vector was derived from an approximately 2972 base pair *Hind*III to *BamHI* restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 2149 base pair *Hind*III to *Acc*I restriction sub-fragment of the SPV *Hind*III restriction fragment M (23). Fragment 2 is an approximately 3006 base pair *Bam*HI to *Pvu*II restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 1571 base pair *Eco*RI to *Stu*I restriction sub-fragment of the PRV *Bam*HI fragment 7 (21). Note that the *Eco*RI site was introduced in to this fragment by PCR cloning. In this procedure the primers described below were used along with a template consisting of a PRV *Bam*HI #7 fragment subcloned into pSP64. The first primer 87.03 (5'- CGCGAATTCGCTCG CAGCGCTATTGGC-3') sits down on the PRV g50 (gD) sequence (26) at approximately amino acid 3 priming toward the 3' end of the gene. The second primer 87.06 (5'-GTAGGAGTGGCTGCTGAAG-3') sits down on the opposite strand at approximately amino acid 174 priming toward the 5' end of the gene. The PCR product may be digested with *Eco*RI and *Sal*I to produce an approximately

509 base pair fragment. The approximately 1049 base pair *Sal*I to *Stu*I sub-fragment of PRV *Bam*HI #7 may then be ligated to the approximately 509 base pair *Eco*RI to *Sal*I fragment to generate the approximately 1558 base pair *Eco*RI to *Stu*I fragment 3. Fragment 4 is an approximately 1484 base pair *Acc*I to *Bgl*II restriction sub-fragment of the SPV *Hind*III fragment M (23).

HOMOLOGY VECTOR 570-91.41. The plasmid 570-91.41 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an *E. coli* ß-galactosidase (lacZ) marker gene and the PRV gIII (gC) gene flanked by SPV DNA. Upstream of the foreign DNA genes is an approximately 2149 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. When this plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß-galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), and the gIII (gC) gene is under the control of a synthetic early late pox promoter (EP1LP2). A detailed description of the plasmid is given in Figures 5A-5D. It may be constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the synthetic DNA sequences indicated in 5A-5D. The plasmid vector was derived from an approximately 2972 base pair *Hind*III to *Bam*HI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair *Bgl*II to *Acc*I restriction sub-fragment of the SPV *Hind*III restriction fragment M (23). Fragment 2 is an approximately 3002 base pair *Bam*HI to *Pvu*II restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 2378 base pair *Nco*I to *Nco*I fragment of plasmid 251-41.A, a subfragment of PRV *Bam*HI #2 and #9.

EcoRI linkers have replaced the NcoI and NcoI sites at the ends of this fragment. Fragment 4 is an approximately 2149 base pair AccI to HindIII restriction sub-fragment of the SPV HindIII fragment M (23). The AccI sites in fragments 1 and 4 have been converted to PstI sites using synthetic DNA linkers.

HOMOLOGY VECTOR 570-91.64. The plasmid 570-91.64 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and the PRV gIII (gC) gene flanked by SPV DNA. Upstream of the foreign DNA genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When this plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß-galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), and the gIII (gC) gene is under the control of a synthetic late early pox promoter (LP2EP2). A detailed description of the plasmid is given in Figures 7A-7D. It may be constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the synthetic DNA sequences indicated in 5 A-5D. The plasmid vector was derived from an approximately 2972 base pair *Hind*III to *Bam*HI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair *Bgl*II to *Acc*I restriction sub-fragment of the SPV *Hind*III restriction fragment M (23). Fragment 2 is an approximately 3002 base pair *Bam*HI to *Pvu*II restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 2378 base pair *Nco*I to *Nco*I fragment of plasmid 251-41.A, a subfragment of PRV *Bam*HI #2 and #9. *Eco*RI linkers have replaced the *Nco*I and *Nco*I sites at the ends of this fragment.

Fragment 4 is an approximately 2149 base pair *Acc*I to *Hind*III restriction sub-fragment of the SPV *Hind*III fragment M (23). The *Acc*I sites in fragments 1 and 4 have been converted to *Pst*I sites using synthetic DNA linkers.

HOMOLOGY VECTOR 727-54.60. The plasmid 727-54.60 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an *E. coli* ß-galactosidase (lacZ) marker gene and the pseudorabies virus (PRV) gII (gB) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß-galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), and the PRV gB gene is under the control of a synthetic late/early pox promoter (LP2EP2). A detailed description of the plasmid is given in Figures 7A-7D. It may be constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences indicated in Figures 7A-7D. The plasmid vector was derived from an approximately 2972 base pair *Hind*III to *Bam*HI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair *Bgl*II to *Acc*I restriction sub-fragment of the SPV *Hind*III restriction fragment M (23). Fragment 2 is an approximately 3500 base pair fragment which contains the coding sequence for the PRV gB gene within the KpnI C fragment of genomic PRV DNA(21). Fragment 2 contains an approximately 53 base pair synthetic fragment containing the amino terminus of the PRV gB gene, an approximately 78 base pair *Sma*I to

Nhe I fragment from the PRV KpnI C genomic fragment, and an approximately 3370 base pair NheI to *Eco*RI fragment from the PRV KpnI C genomic fragment (21). Fragment 3 is an approximately 3010 base pair *Bam*HI to *Pvu*II restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair *Acc*I to *Hind*III subfragment of the SPV *Hind*III fragment M. The *Acc*I sites in fragments 1 and 4 were converted to unique *Not*I sites using *Not*I linkers.

HOMOLOGY VECTOR 751-07.A1. The plasmid 751-07.A1 was used to insert foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and the chicken interferon (cIFN) gene flanked by SPV DNA. When this plasmid was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the ß-galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1) and the cIFN gene is under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1146 base pair BglII to AccI restriction sub-fragment of the SPV HindIII fragment M (23). Fragment 2 is an approximately 577 base pair EcoRI to BglII fragment coding for the cIFN gene (54) derived by reverse transcription and polymerase chain reaction (PCR) (Sambrook, et al., 1989) of RNA ISOLATED FROM CONCANAVALIN A STIMULATED CHICKEN SPLEEN CELLS. The antisense primer (6/94.13) used for reverse transcription

and PCR was 5'-CGACGGATCCGAGGTGCGTTTGGGGCTAAGTGC-3' (SEQ ID NO: 211). The sense primer (6/94.12) used for PCR was 5'-CCACGGATCCAGCACAACGCGAGTCCCACCATGGCT-3' (SEQ ID NO: 212). The BamHI fragment resulting from reverse transcription and PCR was gel purified and used as a template for a second PCR reaction to introduce a unique EcoRI site at the 5' end and a unique BglII site at the 3' end. The second PCR reaction used primer 6/94.22 (5'-CCACGAATTCGATGGCTGTGCCTGCAAGCCCACAG-3'; SEQ ID NO: 213) at the 5' end and primer 6/94.34 (5'-CGAAGATCTGAGGTGCGTTTGGGGCTAAGTGC-3'; SEQ ID NO: 214) at the 3' end to yield an approximately 577 base pair fragment. The DNA fragment contains the coding sequence from amino acid 1 to amino acid 193 of the chicken interferon protein (54) which includes a 31 amino acid signal sequence at the amino terminus and 162 amino acids of the mature protein encoding chicken interferon. Fragment 3 is an approximately 3002 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2156 base pair AccI to HindIII restriction sub-fragment of the SPV HindIII restriction fragment M (23). The AccI site in the SPV homology vector was converted to a unique NotI site.

HOMOLOGY VECTOR 751-56.A1. The plasmid 751-56.A1 was used to insert foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and the chicken myelomonocytic growth factor (cMGF) gene flanked by SPV DNA. When this plasmid was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the ß-galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1) and the cMGF gene is under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining

restriction fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1146 base pair BglII to AccI restriction sub-fragment of the SPV HindIII fragment M (23). Fragment 2 is an approximately 640 base pair EcoRI to BamHI fragment coding for the cMGF gene(55) derived by reverse transcription and polymerase chain reaction (PCR) (Sambrook, et al., 1989) of RNA ISOLATED FROM CONCANAVALIN A STIMULATED CHICKEN SPLEEN CELLS. The antisense primer (6/94.20) used for reverse transcription and PCR was 5'-CGCAGGATCCGGGGCGTCAGAGGCGGGCGAGGTG-3' (SEQ ID NO: 215). The sense primer (5/94.5) used for PCR was 5'-GAGCGGATCCTGCAGGAGGAGACACAGAGCTG-3' (SEQ ID NO: 216). The BamHI fragment derived from PCR was subcloned into a plasmid and used as a template for a second PCR reaction using primer 6/94.16 (5'-GCGCGAATTCCATGTGCTGCCTCACCCCTGTG-3'; SEQ ID NO: 217) at the 5' end and primer 6/94.20 (5'-CGCAGGATCCGGGGCGTCAGAGGCGGGCGAGGTG-3'; SEQ ID NO: 218) at the 3' end to yield an approximately 640 base pair fragment. The DNA fragment contains the coding sequence from amino acid 1 to amino acid 201 of the cMGF protein (55) which includes a 23 amino acid signal sequence at the amino terminus and 178 amino acids of the mature protein encoding cMGF. Fragment 3 is an approximately 3002 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2156 base pair AccI to HindIII restriction sub-fragment of the SPV HindIII restriction fragment M (23). The AccI site in the SPV homology vector was converted to a unique NotI site.

HOMOLOGY VECTOR 752-22.1. The plasmid 752-22.1 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ)

marker gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 855 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1113 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß-galactosidase (lacZ) marker gene is under the control of a swinepox virus O1L gene promoter. The homology vector also contains the synthetic late/early promoter (LP2EP2) into which a second foreign gene is inserted into a unique BamHI or EcoRI site. A detailed description of the plasmid is given in Figures 10A-10D. It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences indicated in Figures 10A-10D. The plasmid vector was derived from an approximately 2519 base pair HindIII to SphI restriction fragment of pSP65 (Promega). Fragment 1 is an approximately 855 base pair sub-fragment of the SPV HindIII restriction fragment M (23) synthesized by polymerase chain reaction using DNA primers 5'-GAA<u>GCATGC</u>CCGTTCTTATCAATAGTTTAGTCGAAAATA-3' and 5'-CATA<u>AGATCT</u>GGCATTGTGTTATTATACTAACAAAAATAAG-3' to produce an 855 base pair fragment with SphI and BglII ends. Fragment 2 is a 3002 base pair *Bam*HI to *Pvu*II fragment derived from plasmid pJF751 (49) containing the *E. coli* lacZ gene. Fragment 3 is an approximately 1113 base pair subfragment of the SPV HindIII fragment M synthesized by polymerase chain reaction using DNA primers 5'-CCGTA<u>GTCGAC</u>AAAGATCGACTTATTAATATGTATGGGATT-3' and 5'-GCCTG<u>AAGCTT</u>CTAGTACAGTATTTACGACTTTTGAAAT-3' to produce an 1113 base pair fragment with SalI and HindIII ends.

HOMOLOGY VECTOR 752-29.33. The plasmid 759.33 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lac Z)

marker gene and an equine herpesvirus type 1 gB gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 855 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 113 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß-galactosidase (lacZ) marker gene is under the control of a swinepox virus 01L gene promoter and the EHV-1 gB gene is under the control of the late/early promoter (LP2EP2). The LP2EP2 promoter-EHV-1 gB gene cassette was inserted into a NotI site of homology vector 738-94.4. Homology vector 752-29.33 was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2519 base pair HindIII to SphI restriction fragment of pSP65 (Promega). Fragment 1 is an approximately 855 base pair sub-fragment of the SPV HindIII restriction fragment M (23) synthesized by polymerase chain reaction using DNA primers 5'-GAAGCATGCCCGTTCTTATCAATAGTTTAGTCGAAAATA-3' and 5'-CATAAGATCTGGCATTGTGTTATTATACTAACAAAAATAAG-3' to produce an 855 base pair fragment with SphI and BglII ends. Fragment 2 is a 3002 base pair *Bam*HI to *Pvu*II fragment derived from plasmid pJF751 (49) containing the *E. coli* lacz gene. Fragment 3 is the product of a PCR reaction (EcoRI to BamHI) and a restriction fragment (BamHI to PmeI) ligated together to yield an EHV-1 gB gene which is an EcoRI to PmeI fragment approximately 2941 base pairs (979 amino acids) in length. The PCR fragment is an approximately 429 base pair fragment having a synthetic EcoRI site at the 5' end of the gene and a natural BamHI site at the 3' end within the BamHI "a" fragment of EHV-1 genomic DNA. The restriction fragment is an approximately 2512 base pair fragment from BamHI to PmeI

within the BamHI "I" fragment of EHV-1 genomic DNA. In the procedure to produce the 5′ end PCR fragment, the primers described below were used with a template consisting of the EHV-1 BamHI "a" and "i" fragments.

The first primer 5/94.3 (5′-CGGAATTCCTCTGGTTCGCCGT-3′) sits down on the EHV-1 gB sequence at amino acid number 2 and introduces an EcoRI site at the 5′ end of the EHV-1 gB gene and an ATG start codon. The second primer 5/94.4 (5′-GACGGTGGATCCGGTAGGCGGT-3′) sits down on the EHV-1 gB sequence at approximately amino acid 144 on the opposite strand to primer 5/94.3 and primes toward the 5′ end of the gene. The PCR product was digested with EcoRI and BamHI to yield a fragment 429 base pairs in length corresponding to the 5′ end of the EHV-1 gB gene. Fragment 3 consists of the products of the PCR reaction (EcoRI to BamHI) and the restriction fragment (BamHI to PmeI) ligated together to yield an EHV-1 gB gene which is an EcoRI to PmeI fragment approximately 2941 base pairs (979 amino acids) in length. Fragment 4 is an approximately 1113 base pair subfragment of the SPV HindIII fragment M synthesized by polymerase chain reaction using DNA primers 5′-CCGTAGTCGACAAAGATCGACTTATTAATATGTATGGGATT-3′ and 5′-GCCTGAAGCTTCTAGTACAGTATTTACGACTTTTGAAAT-3′ to produce an 1113 base pair fragment with SalI and HindIII ends.

HOMOLOGY VECTOR 746-94.1. The plasmid 746-94.1 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and an infectious bovine rhinotracheitis virus glycoprotein E (gE) gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 855 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1113 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV,

a virus containing DNA coding for the foreign genes will result. Note that the ß-galactosidase (lacZ) marker gene is under the control of a swinepox virus O1L gene promoter and the IBRV gE gene is under the control of the late/early promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. A 1250 base pair EcoRI to BamHI fragment coding for amino acids 1 to 417 of the IBRV gE gene (missing 158 amino acids of the carboxy terminal transmembrane region) was inserted into unique EcoRI and BamHI sites of homology vector 752-22.1 (Figures 10A-10D). The 1250 base pair EcoRI to BamHI fragment was synthesized by polymerase chain reaction (15) using IBRV (Cooper) genomic DNA as a template and primer 10/94.23 (5'-GGGGAATTCAATGCAACCCACCGCGCCGCCCC-3'; SEQ ID NO: 219) at the 5' end of the IBRV gE gene (amino acid 1) and primer 10/94.22 (5'-GGGGGATCCTAGGGCGCGCCCGCCGGCTCGCT-3'; SEQ ID NO: 220) at amino acid 417 of the IBRV gE gene.

HOMOLOGY VECTOR 767-67.3. The plasmid 767-67.3 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and an bovine viral diarrhea virus glycoprotein 53 (BVDV gp53) gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 855 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1113 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß-galactosidase (lacZ) marker gene is under the control of a swinepox virus O1L gene promoter and the BVDV gp53 gene is under the control of the late/early promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30),

by joining restriction fragments from the following sources with the synthetic DNA sequences. A 1187 base pair BamHI fragment coding for the BVDV gp53 was inserted into the unique BamHI sites of homology vector 752-22.1 (Figures 10A-10D). The 1187 base pair BamHI fragment was synthesized by polymerase chain reaction (15) as described in CLONING OF BOVINE VIRAL DIARRHEA VIRUS gp48 AND gp53 GENES.

HOMOLOGY VECTOR 771-55.11. The plasmid 771-55.11 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and an bovine viral diarrhea virus glycoprotein 48 (BVDV gp48) gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 855 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1113 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß-galactosidase (lacZ) marker gene is under the control of a swinepox virus O1L gene promoter and the BVDV gp48 gene is under the control of the late/early promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. A 678 base pair BamHI fragment coding for the BVDV gp48 was inserted into the unique BamHI sites of homology vector 752-22.1 (Figures 10A-10D). The 678 base pair BamHI fragment was synthesized by polymerase chain reaction (15) as described in CLONING OF BOVINE VIRAL DIARRHEA VIRUS gp48 AND gp53 GENES.

PLASMID 551-47.23. The plasmid 551-47.23 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates the E. coli ß-glucuronidase (ß-glu) marker

gene under the control of a late/early pox promoter (LP2EP2). It is useful to insert the marker gene into sites in the SPV genome to produce a recombinant swinepox virus. It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources. The plasmid vector was derived from an approximately 3005 base pair HindIII restriction fragment of pSP65 (Promega). Fragment 1 is an approximately 1823 base pair EcoRI to SmaI fragment of the plasmid pRAJ260 (Clonetech). Note that the EcoRI and SmaI sites were introduced by PCR cloning. Plasmid 551-47.23 was used to make recombinant swinepox viruses S-SPV-059, S-SPV-060, S-SPV-061, and S-SPV-062.

HOMOLOGY VECTOR 779-94.31. The plasmid 779-94.31 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and the pseudorabies virus (PRV) gB (gII) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 538 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1180 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1) and the PRV gB gene is under the control of a synthetic late/early pox promoter (LP2EP2). A detailed description of the plasmid is given in Figures 12A-12E. It was constructed utilizing standard recombinant DNA techniques (22, and 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2986 base pair HindIII to PstI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 542 base pair HindIII to BglII restriction sub-fragment of the SPV HindIII restriction

fragment M (23). Fragment 2 is an approximately 3500 base pair fragment which contains the coding sequence for the PRV gB gene within the KpnI C fragment of genomic PRV DNA (21). Fragment 2 contains an approximately 53 base pair synthetic fragment containing the amino terminus of the PRV gB gene, an approximately 78 base pair SmaI to Nhe I fragment from the PRV KpnI C genomic fragment, and an approximately 3370 base pair NheI to EcoRI fragment from the PRV KpnI C genomic fragment (21). Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 1180 base pair BglII to PstI subfragment of the SPV HindIII fragment M. The BglII sites in fragments 1 and 4 were converted to unique HindIII sites using HindIII linkers.

HOMOLOGY VECTOR 789-41.7. The plasmid 789-41.7 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene, the pseudorabies virus (PRV) gB (gII) gene and the PRV gD (g50) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the PRV gB gene is under the control of a synthetic late/early pox promoter (LP2EP2), and the PRV gD gene is under the control of a synthetic early/late pox promoter (EP1LP2). A detailed description of the plasmid is given in Figures 13A-13D. It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid

vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 1552 base pair subfragment of the PRV BamHI #7 fragment which contains the coding sequence of the PRV gD gene from amino acid 3 to amino acid 279. The EcoRI site and the ATG translation start codon are derived from a polymerase chain reaction using a 5′ primer with an EcoRI site. The StuI site at the 3′ end is normally within the PRV gI gene 3′ to the PRV gD gene. The entire open reading frame beginning at the EcoRI site codes for 405 amino acids. Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 3500 base pair fragment which contains the coding sequence for the PRV gB gene within the KpnI C fragment of genomic PRV DNA(21). Fragment 4 contains an approximately 53 base pair synthetic fragment containing the amino terminus of the PRV gB gene, an approximately 78 base pair SmaI to Nhe I fragment from the PRV KpnI C genomic fragment, and an approximately 3370 base pair NheI to EcoRI fragment from the PRV KpnI C genomic fragment (21). Fragment 5 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 6 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique PstI sites using PstI linkers. The NdeI sites in fragments 3 and 6 were converted to unique HindIII sites using HindIII linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104;) of the SPV HindIII M fragment has been deleted which would span SPV fragments 3 and 6.

HOMOLOGY VECTOR 789-41.27. The plasmid 789-41.27 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene, the pseudorabies virus (PRV) gB (gII) gene and the PRV gC (gIII) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the PRV gB gene is under the control of a synthetic late/early pox promoter (LP2EP2), and the PRV gC gene is under the control of a synthetic early/late pox promoter (EP1LP2). A detailed description of the plasmid is given in Figures 14A-14D. It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences indicated. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1560 base pair HindIII to NdeI subfragment of the SPV HindIII fragment M. Fragment 2 is an approximately 3500 base pair fragment which contains the coding sequence for the PRV gB gene within the KpnI C fragment of genomic PRV DNA(21). Fragment 2 contains an approximately 53 base pair synthetic fragment containing the amino terminus of the PRV gB gene, an approximately 78 base pair SmaI to Nhe I fragment from the PRV KpnI C genomic fragment, and an approximately 3370 base pair NheI to EcoRI fragment from the PRV KpnI C genomic fragment (21). Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 48 base pair AccI to NdeI subfragment of

the SPV HindIII M fragment. Fragment 5 is an approximately 2378 base pair NcoI to NcoI fragment of plasmid 251-41.A, a subfragment of PRV BamHI #2 and #9. EcoRI linkers have replaced the NcoI sites at the ends of the fragment. Fragment 6 is an approximately 1484 base pair AccI to BglII restriction sub-fragment of the SPV HindIII restriction fragment M (23). The NdeI sites in fragments 1 and 4 were converted to unique HindIII sites using HindIII linkers. The AccI site in fragments 4 and 6 were converted to unique PstI sites using PstI linkers. An approximately 545 base pair NdeI to NdeI (Nucleotides 1560 to 2104;) subfragment of the SPV HindIII M fragment has been deleted which would span SPV fragments 4 and 6.

HOMOLOGY VECTOR 789-41.47. The plasmid 789-41.47 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene, the pseudorabies virus (PRV) gC (gIII) gene and the PRV gD (g50) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the PRV gC gene is under the control of a synthetic early/late pox promoter (EP1LP2), and the PRV gD gene is under the control of a synthetic early/late pox promoter (EP1LP2). A detailed description of the plasmid is given in Figures 15A-15D. It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega).

Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 1552 base pair subfragment of the PRV BamHI #7 fragment which contains the coding sequence of the PRV gD gene from amino acid 3 to amino acid 279. The EcoRI site and the ATG translation start codon are derived from a polymerase chain reaction using a 5′ primer with an EcoRI site. The StuI site at the 3′ end is normally within the PRV gI gene 3′ to the PRV gD gene. The entire open reading frame beginning at the EcoRI site codes for 405 amino acids. Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 5 is an approximately 2378 base pair NcoI to NcoI fragment of plasmid 251-41.A, a subfragment of PRV BamHI #2 and #9. EcoRI linkers have replaced the NcoI sites at the ends of the fragment. Fragment 6 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique PstI sites using PstI linkers. The NdeI sites in fragments 3 and 6 were converted to unique HindIII sites using HindIII linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104;) of the SPV HindIII M fragment has been deleted which would span SPV fragments 3 and 6.

HOMOLOGY VECTOR 789-41.73. The plasmid 789-41.73 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene, the pseudorabies virus (PRV) gB (gII) gene, the PRV gC (gIII) gene and the PRV gD (g50) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1560

base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the PRV gB gene is under the control of a synthetic late/early pox promoter (LP2EP2), the PRV gC gene is under the control of a synthetic early/late promoter (EP1LP2), and the PRV gD gene is under the control of a synthetic late/early pox promoter (LP2EP2). A detailed description of the plasmid is given in Figures 16A-16E. It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 1552 base pair subfragment of the PRV BamHI #7 fragment which contains the coding sequence of the PRV gD gene from amino acid 3 to amino acid 279. The EcoRI site and the ATG translation start codon are derived from a polymerase chain reaction using a 5′ primer with an EcoRI site. The StuI site at the 3′ end is normally within the PRV gI gene 3′ to the PRV gD gene. The entire open reading frame beginning at the EcoRI site codes for 405 amino acids. Fragment 3 is an approximately 2378 base pair NcoI to NcoI fragment of plasmid 251-41.A, a subfragment of PRV BamHI #2 and #9. EcoRI linkers have replaced the NcoI sites at the ends of the fragment. Fragment 4 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 5 is an approximately 3500 base pair fragment which contains the coding sequence for the PRV gB gene within the KpnI C fragment of genomic PRV DNA(21). Fragment 5 contains an approximately 53 base pair

synthetic fragment containing the amino terminus of the PRV gB gene, an approximately 78 base pair SmaI to Nhe I fragment from the PRV KpnI C genomic fragment, and an approximately 3370 base pair NheI to EcoRI fragment from the PRV KpnI C genomic fragment (21). Fragment 6 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 7 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique PstI sites using PstI linkers. The NdeI sites in fragments 3 and 6 were converted to unique HindIII sites using HindIII linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104;) of the SPV HindIII M fragment has been deleted which would span SPV fragments 3 and 6.

HOMOLOGY VECTOR 791-63.19. The plasmid 791-63.19 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequence. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an

approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 2149 base pair AccI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique NotI sites using NotI linkers.

HOMOLOGY VECTOR 791-63.41. The plasmid 791-63.41 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 2149 base pair AccI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique NotI sites using NotI linkers.

HOMOLOGY VECTOR 796-18.9. The plasmid 796-18.9 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ)

marker gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß galactosidase (lacZ) marker gene is under the control of a synthetic early pox promoter (EP1). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 2149 base pair AccI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique NotI sites using NotI linkers.

HOMOLOGY VECTOR 783-39.2. The plasmid 783-39.2 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and an bovine viral diarrhea virus glycoprotein 53 (BVDV gp53) gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß-galactosidase (lacZ) marker gene is under the control of a late promoter (LP1) and the

BVDV gp53 gene is under the control of the late/early promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 1187 base pair BamHI fragment coding for the BVDV gp53. The 1187 base pair BamHI fragment was synthesized by polymerase chain reaction (15) as described in CLONING OF BOVINE VIRAL DIARRHEA VIRUS gp48 AND gp53 GENES. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair AccI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 4 were converted to unique NotI sites using NotI linkers.

HOMOLOGY VECTOR 749-75.78. The plasmid 749-75.78 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and the infectious bursal disease virus (IBDV) polymerase gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1) and the IBDV polymerase gene is under the control of a synthetic late/early promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques

(22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 2700 EcoRI to AscI restriction fragment synthesized by cDNA cloning and polymerase chain reaction (PCR) from an IBDV RNA template. cDNA and PCR primers (5'-CAC<u>GAATTC</u>TGACATTTTCAACAGTCCACAGGCGC-3'; 12/93.4) and 5'-GCTGTTGGACATCACGGGCCAGG-3'; 9/93.28) were used to synthesize an approximately 1400 base pair EcoRI to BclI fragment at the 5' end of the IBDV polymerase gene. cDNA and PCR primers (5'-ACCCGGAACATATGGTCAGCTCCAT-3'; 12/93.2) and 5'-<u>GGCGCGCC</u>AGGCGAAGGCCGGGGATACGG-3'; 12/93.3) were used to synthesize an approximately 1800 base pair BclI to AscI fragment at the 3' end of the IBDV polymerase gene. The two fragments were ligated at the BclI site to form the approximately 2700 base pair EcoRI to BclI fragment. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair AccI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 4 were converted to unique NotI sites using NotI linkers.

HOMOLOGY VECTOR 761-75.B18. The plasmid 761-75.B18 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lac Z) marker gene and a feline immunodeficiency virus (FIV) protease (gag) gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 855 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1113 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV,

a virus containing DNA coding for the foreign genes will result. Note that the ß galactosidase (lacZ) marker gene is under the control of a swinepox virus 01L gene promoter and the FIV gag gene is under the control of the late/early promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2519 base pair HindIII to SphI restriction fragment of pSP65 (Promega). Fragment 1 is an approximately 855 base pair sub-fragment of the SPV HindIII restriction fragment M (23) synthesized by polymerase chain reaction using DNA primers 5' GAAGCATGCCCGTTCTTATCAATAGTTTAGTCGAAAATA-3' and 5'-CATAAGATCTGGCATTGTGTTATTATACTAACAAAAATAAG-3' to produce an 855 base pair fragment with SphI and BglII ends. Fragment 2 is a 3002 base pair BamHI to PvuII fragment derived from plasmid pJF751 (49) containing the E. coli lacZ gene. Fragment 3 is an approximately 1878 base pair EcoRI to BglII restriction fragment synthesized by polymerase chain reaction (PCR) using cDNA from the FIV (PPR strain) (61). The primer (5' GCGT<u>GAATTC</u>GGGGAATGGACAGGGGCGAGAT-3'; 11/94.9) synthesizes from the 5' end of the FIV gag gene, introduces an EcoRI site at the 5' end of the gene and an ATG start codon. The primer (5'-GAGCC<u>AGATCT</u>GCTCTTTTTACTTTCCC-3'; 11/94.10) synthesizes from the 3' end of the FIV gag gene. The PCR product was digested with EcoRI and BglII to yield a fragment 1878 base pairs in length corresponding to the FIV gag gene. Fragment 4 is an approximately 1113 base pair subfragment of the SPV HindIII fragment M synthesized by polymerase chain reaction using DNA primers 5'-CCGTAGTCGACAAAGATCGACTTATTAATATGTATGGGATT-3' and 5' GCCTGAAGCTTCTAGTACAGTATTTACGACTTTTGAAAT-3' to produce an 1113 base pair fragment with SalI and HindIII ends.

HOMOLOGY VECTOR 781-84.C11. The plasmid 781-84.C11 was used to insert foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and the feline immunodeficiency virus (FIV) envelope (env) gene flanked by SPV DNA. When this plasmid was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the ß galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1) and the FIV env gene is under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII fragment M (23). Fragment 3 is an approximately 2564 base pair *Bam*HI to *Bam*HI fragment of the FIV *env* gene (61) synthesized by CLONING WITH THE POLYMERASE CHAIN REACTION. The template for the PCR reaction was FIV strain PPR genomic cDNA (61). The upstream primer 10/93.21 (5'-GCCCGGATCCTATGGCAGAAGGGTTTGCAGC-3';) was synthesized corresponding to the 5' end of the FIV *env* gene starting at nucleotide 6263 of FIV strain PPR genomic cDNA, and the procedure introduced a *Bam*HI site at the 5' end. The *Bam*HI site was destroyed during the cloning of the PCR fragment. The downstream primer 10/93.20 (5'-CCGTGGATCCGGCACTCCATCATTCCTCCTC-3';) was synthesized corresponding to the 3' end of the FIV env gene starting at nucleotide 8827 of FIV PPR genomic cDNA, and the procedure introduced a *BamHI* site at the 3' end. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair AccI to

HindIII restriction sub-fragment of the SPV HindIII restriction fragment M (23). The AccI site in the SPV homology vector was converted to a unique NotI site.

EXAMPLES

Example 1

Homology Vector 515-85.1. The homology vector 515-85.1 is a plasmid useful for the insertion of foreign DNA into SPV. Plasmid 515-85.1 contains a unique *Acc*I restriction site into which foreign DNA may be cloned. A plasmid containing such a foreign DNA insert may be used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV to generate a SPV containing the foreign DNA. For this procedure to be successful it is important that the insertion site (*Acc*I) be in a region non-essential to the replication of the SPV and that the site be flanked with swinepox virus DNA appropriate for mediating homologous recombination between virus and plasmid DNAs. *Acc*I site in homology vector 515-85.1 is used to insert foreign DNA into at least three recombinant SPV (see examples 2-4).

In order to define an appropriate insertion site, a library of SPV *Hind*III restriction fragments was generated. Several of these restriction fragments (*Hind*III fragments G, J, and M see Figures 1A-1B) were subjected to restriction mapping analysis. Two restriction sites were identified in each fragment as potential insertion sites. These sites included *Hpa*I and *Nru*I in fragment G, *Bal*I and *Xba*I in fragment J, and *Acc*I and *Pst*I in fragment M. A ß-galactosidase (lacZ) marker gene was inserted in each of the potential sites. The resulting plasmids were utilized in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The generation of recombinant virus was determined by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-GALACTOSIDASE ASSAYS. Four of the six sites were found to generate recombinant virus, however the ability of each of these viruses to be purified away from the parental SPV varied

greatly. In one case virus could not be purified above the level of 1%, in another case virus could not be purified above the level of 50%, and in a third case virus could not be purified above the level of 90%. The inability to purify these viruses indicates instability at the insertion site. This makes the corresponding sites inappropriate for insertion of foreign DNA. However the insertion at one site, the <u>Acc</u>I site of Homology vector 515-85.1, resulted in a virus which was easily purified to 100% (see example 2), clearly defining an appropriate site for the insertion of foreign DNA.

The homology vector 515-85.1 was further characterized by DNA sequence analysis. Two regions of the homology vector were sequenced. The first region covers a 599 base pair sequence which flanks the unique *Acc*I site. The second region covers the 899 base pairs upstream of the unique *HindIII site. The sequence of the first region codes for an open reading frame (ORF) which shows homology to amino acids 1 to 115 of the vaccinia virus (VV) O1L open reading frame identified by Goebel <u>et al</u>, 1990 (see Figures 2A-2C). The sequence of the second region codes for an open reading frame which shows homology to amino acids 568 to 666 of the same vaccinia virus O1L open reading frame (see Figures 2A-2C). These data suggest that the AccI* site interrupts the presumptive VV O1L-like ORF at approximately amino acid 41, suggesting that this ORF codes for a gene non-essential for SPV replication. Goebel *et al*. suggest that the VV O1L ORF contains a leucine zipper motif characteristic of certain eukaryotic transcriptional regulatory proteins, however they indicate that it is not known whether this gene is essential for virus replication.

The DNA sequence located upstream of the VV 01L-like ORF would be expected to contain a swinepox viral promoter.

This swinepox viral promoter will be useful as the control element of foreign DNA introduced into the swinepox genome.

Example 2

S-SPV-003

S-SPV-003 is a swinepox virus that expresses a foreign gene. The gene for *E.coli* ß-galactosidase (lacZ gene) was inserted into the SPV 515-85.1 ORF. The foreign gene (lacZ) is under the control of a synthetic early/late promoter (EP1LP2).

S-SPV-003 was derived from S-SPV-001 (Kasza strain). This was accomplished utilizing the homology vector 520-17.5 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-GALACTOSIDASE (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-003. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable and expressing the foreign gene. The assays described here were carried out in VERO cells as well as EMSK cells, indicating that VERO cells would be a suitable substrate for the production of SPV recombinant vaccines. S-SPV-003 has been deposited with the ATCC under Accession No. VR 2335.

Example 3

S-SPV-008

S-SPV-008 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* ß-galactosidase (lacZ gene) and the gene for pseudorabies virus (PRV) g50 (gD) (26) were inserted into the SPV 515-85.1 ORF. The lacZ gene is under the control of a synthetic late promoter (LP1) and the g50 (gD) gene is under the control of a synthetic early/late promoter (EP1LP2).

S-SPV-008 was derived from S-SPV-001 (Kasza strain). This was accomplished utilizing the homology vector 538-46.16 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-GALACTOSIDASE (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-008. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable and expressing the marker gene.

S-SPV-008 was assayed for expression of PRV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Swine anti-PRV serum was shown to react specifically with S-SPV-008 plaques and not with S-SPV-009 negative control plaques. All S-SPV-008 observed plaques reacted with the swine antiserum indicating that the virus was stably expressing the PRV foreign gene. The black plaque assay was also performed on unfixed monolayers. The SPV plaques on the unfixed

monolayers also exhibited specific reactivity with swine anti-PRV serum indicating that the PRV antigen is expressed on the infected cell surface.

To confirm the expression of the PRV g50 (gD) gene product, cells were infected with SPV and samples of infected cell lysates were subjected to SDS-polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. The swine anti-PRV serum was used to detect expression of PRV specific proteins. The lysate from S-SPV-008 infected cells exhibits a specific band of approximately 48 kd, the reported size of PRV g50 (gD) (35).

PRV g50 (gD) is the g50 (gD) homologue of HSV-1 (26). Several investigators have shown that VV expressing HSV-1 g50 (gD) will protect mice against challenge with HSV-1 (6 and 34). Therefore the S-SPV-008 should be valuable as a vaccine to protect swine against PRV disease.

It is anticipated that several other PRV glycoproteins will be useful in the creation of recombinant swinepox vaccines to protect against PRV disease. These PRV glycoproteins include gII (28), gIII (27), and gH (19). The PRV gIII coding region has been engineered behind several synthetic pox promoters. The techniques utilized for the creation of S-SPV-008 will be used to create recombinant swinepox viruses expressing all four of these PRV glycoprotein genes. Such recombinant swinepox viruses will be useful as vaccines against PRV disease. Since the PRV vaccines described here do not express PRV gX or gI, they would be compatible with current PRV diagnostic tests (gX HerdChek®, gI HerdChek® and ClinEase®) which are utilized to distinguish vaccinated animals from infected animals. S-SPV-008 has been deposited with the ATCC under Accession No. VR 2339.

Example 6

S-SPV-013

S-SPV-013 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* ß-galactosidase (lacZ) and the gene for pseudorabies virus gIII (gC) were inserted into the unique *Pst*I restriction site (*Pst*I linkers inserted into a unique *Acc*I site) of the homology vector 570-33.32. The lacZ gene is under the control of the synthetic late promoter (LP1) and the PRV gIII (gC) gene is under the control of the synthetic late early promoter (LP2EP2).

S-SPV-013 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 570-91.64 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-013. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-013 was assayed for expression of PRV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal goat anti-PRV gIII (gC) antibody was shown to react specifically with S-SPV-013 plaques and not with S-SPV-001 negative control plaques. All S-SPV-013 observed plaques reacted with the swine anti-PRV serum indicating that the virus was stably

expressing the PRV foreign gene. The assays described here were carried out in EMSK and VERO cells, indicating that EMSK cells would be a suitable substrate for the production of SPV recombinant vaccines.

To confirm the expression of the PRV gIII (gC) gene product, cells were infected with SPV and samples of infected cell lysates were subjected to SDS-polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. Polyclonal goat anti-PRV gIII (gC) antibody was used to detect expression of PRV specific proteins. The lysate from S-SPV-013 infected cells exhibits two specific bands which are the reported size of PRV gIII (gC) (37)—a 92 kd mature form and a 74 kd pre-Golgi form.

Recombinant-expressed PRV gIII (gC) has been shown to elicit a significant immune response in mice and swine (37, 38). Furthermore, when gIII (gC) is coexpressed with gII (gB) or g50 (gD), significant protection from challenge with virulent PRV is obtained. (39) Therefore S-SPV-013 is valuable as a vaccine to protect swine against PRV disease. Since the PRV vaccines described here do not express PRV gX or gI, they would be compatible with current PRV diagnostic tests (gX HerdChek®, gI HerdChek® and ClinEase®) which are utilized to distinguish vaccinated animals from infected animals. S-SPV-013 has been deposited with the ATCC under Accession No. 2418.

Protection against Aujeszky's disease using recombinant swinepox virus vaccines S-SPV-008 and S-SPV-013.

A vaccine containing S-SPV-008 and S-SPV-013 (1 x 10^6PFU/ml) (2ml of a 1:1 mixture of the two viruses) was given to two groups of pigs (5 pigs per group) by intradermal inoculation or by oral/pharyngeal spray. A

control group of 5 pigs received S-SPV-001 by both intradermal and oral/pharyngeal inoculation. Pigs were challenged three weeks post-vaccination with virulent PRV, strain 4892, by intranasal inoculation. The table presents a summary of clinical responses. The data support an increase in protection against Aujeszky's disease in the S-SPV-008/S-SPV-013 vaccinates compared to the S-SPV-001 vaccinate controls.

Vaccine	Route of inoculation	Post-challenge Respiratory Signs: (# with signs/ total number)	Post-challenge CNS signs: (# with signs/ total number)	Post-challenge Group average: (Days of clinical signs)
S-SPV-008 + S-SPV-013	Intradermal	3/5	0/5	2.6
S-SPV-008 + S-SPV-013	Oral/ pharyngeal	3/5	0/5	2.2
S-SPV-001 (Control)	Intradermal + Oral/ Pharyngeal	5/5	2/5	7.8

Example 7

S-SPV-015

S-SPV-015 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* ß-galactosidase (lacZ) and the gene for pseudorabies virus (PRV) gII (gB) were inserted into the SPV 617-48.1 ORF (a unique *Not*I restriction site has replaced a unique *Acc*I restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the PRV gB gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-015 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 727-54.60 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-015. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-015 was assayed for expression of PRV-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-PRV serum was shown to react specifically with S-SPV-015 plaques and not with S-SPV-001 negative control plaques. All S-SPV-015 observed plaques reacted with the antiserum indicating that the virus was stably expressing the PRV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

To confirm the expression of the PRV gII gene product, cells were infected with SPV-015 and samples of infected cell lysates were subjected to SDS-polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. Polyclonal swine anti-PRV serum was used to detect expression of PRV specific proteins. The lysate from S-SPV-015 infected cells exhibited bands corresponding to 120 kd, 67 kd and 58 kd, which are the expected size of the PRV gII glycoprotein.

S-SPV-015 is useful as a vaccine in swine against pseudorabies virus. A superior vaccine is formulated by combining S-SPV-008 (PRV g50), S-SPV-013 (PRV gIII), and S-SPV-015 for protection against pseudorabies in swine.

Therefore S-SPV-015 should be valuable as a vaccine to protect swine against PRV disease. Since the PRV vaccines described here do not express PRV gX or gI, they would be compatible with current PRV diagnostic tests (gX HerdChek®, gI HerdChek® and ClinEase®) which are utilized to distinguish vaccinated animals from infected animals. S-SPV-015 has been deposited with the ATCC under Accession No. 2466.

Example 8

Recombinant swinepox virus expressing more than one pseudorabies virus (PRV) glycoproteins, which can elicit production of neutralizing antibodies against pseudorabies virus, is constructed in order to obtain a recombinant swinepox virus with enhanced ability to protect against PRV infection than that which can be obtained by using a recombinant swinepox virus expressing only one of those PRV glycoproteins.

There are several examples of such recombinant swinepox virus expressing more than one PRV glycoproteins: a recombinant swinepox virus expressing PRV g50 (gD) and gIII (gC), a recombinant swinepox virus expressing PRV g50 (gD) and gII (gB); a recombinant swinepox virus expressing PRV gII (gB) and gIII (gC); and a recombinant swinepox virus expressing PRV g50 (gD), gIII (gC) and gII (gB). Each of the viruses cited above is also engineered to contain and express *E. coli* ß-galactosidase (lac Z) gene, which will facilitate the cloning of the recombinant swinepox virus.

Listed below are three examples of a recombinant swinepox virus expressing PRV g50 (gD), PRV gIII (gC), PRV gII (gB) and *E. coli* ß-galactosidase (lacZ):

a) Recombinant swinepox virus containing and expressing PRV g50 (gD) gene, PRV gIII (gC) gene, PRV gII (gB) gene and lacZ gene. All four genes are inserted into the unique *Acc*I restriction endonuclease site within the *Hind*III M fragment of the swinepox virus genome. PRV g50 (gD) gene is under the control of a synthetic early/late promoter (EP1LP2), PRV gIII (gC) gene is under the control of a synthetic early promoter (EP2), PRV gII (gB) gene is under the control of a synthetic late/early promoter (LP2EP2) and lacZ gene is under the control of a synthetic late promoter (LP1).

b) Recombinant swinepox virus containing and expressing PRV g50 (gD) gene, PRV gIII (gC) gene, PRV gII (gB) gene and lacZ gene. All four genes are inserted into the unique *Acc*I restriction endonuclease site within the *Hind*III M fragment of the swinepox virus genome. PRV g50 (gD) gene is under the control of a synthetic early/late promoter (EP1LP2), PRV gIII (gC) gene is under the control of a synthetic early/late promoter (EP1LP2), PRV gII (gB) gene is under the control of a synthetic late/early promoter (LP2EP2) and lacZ gene is under the control of a synthetic late promoter (LP1).

c) Recombinant swinepox virus containing and expressing PRV g50 (gD) gene, PRV gIII (gC) gene, PRV gII (gB) gene and lacZ gene. All four genes are inserted into the unique *Acc*I restriction endonuclease site within the *Hind*III M fragment of the swinepox virus genome. PRV g50 (gD) gene is under the control of a synthetic early/late promoter

(EP1LP2), PRV gIII (gC) gene is under the control of a synthetic late/early promoter (LP2EP2), PRV gII (gB) gene is under the control of a synthetic late/early promoter (LP2EP2) and lacZ gene is under the control of a synthetic late promoter (LP1).

Protection against Aujeszky's disease using recombinant swinepox virus vaccines S-SPV-008, S-SPV-013 and S-SPV-015.

A vaccine containing S-SPV-008, S-SPV-013, or S-SPV-015 (2 ml of 1 X 10^7 PFU/ml of the virus) or a mixture of S-SPV-008, S-SPV-013, and S-SPV-015 (2ml of a 1:1:1 mixture of the three viruses; 1 X 10^7 PFU/ml) was given to four groups of pigs (5 pigs per group) by intramuscular inoculation. A control group of 5 pigs received S-SPV-001 by intramuscular inoculation. Pigs were challenged four weeks post-vaccination with virulent PRV, strain 4892, by intranasal inoculation. The pigs were observed daily for 14 days for clinical signs of pseudorabies, and the table presents a summary of clinical responses.The data show that pigs vaccinated with S-SPV-008, S-SPV-013, or S-SPV-015 had partial protection and pigs vacinated with the combination vaccine S-SPV-008/S-SPV-013/S-SPV-015 had complete protection against Aujeszky's disease caused by pseudorabies virus compared to the S-SPV-001 vaccinate controls.

Vaccine	Route of inoculation	Post-challenge Respiratory Signs: (# with signs/ total number)	Post-challenge CNS signs: (# with signs/ total number)	Post-challenge Group average: (Days of clinical signs)
S-SPV-008	Intramuscular	2/5	2/5	2.0
S-SPV-013	Intramuscular	1/5	0/5	0.4

S-SPV-015	Intramuscular	3/5	0/5	1.0
S-SPV-008 + S-SPV-013 + S-SPV-015	Intramuscular	0/5	0/5	0.0
S-SPV-001 (Control)	Intramuscular	5/5	2/5	3.6

Example 17

The development of vaccines utilizing the swinepox virus to express antigens from various disease causing microorganisms can be engineered.

TRANSMISSIBLE GASTROENTERITIS VIRUS

The major neutralizing antigen of the transmissible gastroenteritis virus (TGE), glycoprotein 195, for use in the swinepox virus vector has been cloned. The clone of the neutralizing antigen is disclosed in U.S. Serial No. 078,519, filed July 27, 1987. It is contemplated that the procedures that have been used to express PRV g50 (gD) in SPV and are disclosed herein are applicable to TGE.

PORCINE PARVOVIRUS

The major capsid protein of the porcine (swine) parvovirus (PPV) was cloned for use in the swinepox virus vector. The clone of the capsid protein is disclosed in U.S. Patent No. 5,068,192 issued November 26, 1991. It is contemplated that the procedures that have been used to express PRV g50 (gD) in SPV and are disclosed herein are applicable to PPV.

SWINE ROTAVIRUS

The major neutralizing antigen of the swine rotavirus, glycoprotein 38, was cloned for use in the swinepox virus vector. The clone of glycoprotein 38 is disclosed

in U.S. Patent No. 5,068,192 issued November 26, 1991. It is contemplated that the procedures that have been used to express PRV g50 (gD) in SPV and are disclosed herein are applicable to SRV.

HOG CHOLERA VIRUS

The major neutralizing antigen of the bovine viral diarrhea (BVD) virus was cloned as disclosed in U.S. Serial No. 225,032, filed July 27, 1988. Since the BVD and hog cholera viruses are cross protective (31), the BVD virus antigen has been targeted for use in the swinepox virus vector. It is contemplated that the procedures that have been used to express PRV g50 (gD) in SPV and are disclosed herein are applicable to BVD virus.

SERPULINA HYODYSENTERIAE

A protective antigen of *Serpulina hyodysenteriae* (3), for use in the swinepox virus vector has been cloned. It is contemplated that the procedures that have been used to express PRV g50 in SPV and are disclosed herein are also applicable to *Serpulina hyodysenteriae*.

Antigens from the following microorganisms may also be utilized to develop animal vaccines: swine influenza virus, foot and mouth disease virus, African swine fever virus, hog cholera virus, *Mycoplasma hyopneumoniae*, porcine reproductive and respiratory syndrome/swine infertility and respiratory syndrome (PRRS/SIRS).

Antigens from the following microorganisms may also be utilized for animal vaccines: 1) canine - herpesvirus, canine distemper, canine adenovirus type 1 (hepatitis), adenovirus type 2 (respiratory disease), parainfluenza, *Leptospira canicola*, icterohemorragia, parvovirus, coronavirus, *Borrelia burgdorferi*, canine herpesvirus,

Bordetella bronchiseptica, Dirofilaria immitis (heartworm) and rabies virus. 2) Feline - Fiv gag and env, feline leukemia virus, feline immunodeficiency virus, feline herpesvirus, feline infectious peritonitis virus, canine herpesvirus, canine coronavirus, canine parvovirus, parasitic diseases in animals (including *Dirofilaria immitis* in dogs and cats), equine infectious anemia, *Streptococcus equi*, coccidia, emeria, chicken anemia virus, *Borrelia bergdorferi*, bovine coronavirus, *Pasteurella haemolytica*.

Example 24

Homology Vector 738-94.4

Homology Vector 738-94.4 is a swinepox virus vector that expresses one foreign gene. The gene for *E. coli* ß-galactosidase (lacZ) was inserted into the the O1L open reading frame. The lacZ gene is under the control of the O1L promoter. The homology vector 738-94.4 contains a deletion of SPV DNA from nucleotides 1679 to 2452 (SEQ ID NO: 189) which deletes part of the O1L ORF.

The upstream SPV sequences were synthesized by polymerase chain reaction using DNA primers 5'-GAAGCATGCCCGTTCTTATCAATAGTTTAGTCGAAAATA-3' and 5'-CATAAGATCTGGCATTGTGTTATTATACTAACAAAAATAAG-3' to produce an 855 base pair fragment with BglII and SphI ends. The O1L promoter is present on this fragment. The downstream SPV sequences were synthesized by polymerase chain reaction using DNA primers 5'-CCGTAGTCGACAAAGATCGACTTATTAATATGTATGGGATT-3' and 5'-GCCTGAAGCTTCTAGTACAGTATTTACGACTTTTGAAAT-3' to produce an 1113 base pair fragment with SalI and HindIII ends.

A recombinant swinepox virus was derived utilizing homology vector 738-94.4 and S-SPV-001 (Kasza strain) in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification is the recombinant virus. This virus is assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign gene. Recombinant swinepox viruses derived from homology vector 738-94.4 are utilized as an expression vector to express foreign antigens and as a vaccine to raise a protective immune response in animals to foreign genes expressed by the recombinant swinepox virus. Other promoters in addition to the O1L promoter are inserted into the deleted region including LP1, EP1LP2, LP2EP2, HCMV immediate early, and one or more foreign genes are expressed from these promoters.

Example 24B

Homology Vector 752-22.1 is a swinepox virus vector that is utilized to express two foreign genes. The gene for *E. coli* ß-galactosidase (lacZ) was inserted into the the O1L open reading frame. The lacZ gene is under the control of the O1L promoter. A second foreign gene is expressed from the LP2EP2 promoter inserted into an EcoRI or BamHI site following the LP2EP2 promoter sequence. The homology vector 752-22.1 contains a deletion of SPV DNA from nucleotides 1679 to 2452 (SEQ ID NO: 189) which deletes part of the O1L ORF. The homology vector 752-22.1 was derived from homology

vector 738-94.4 by insertion of the LP2EP2 promoter fragment (see Materials and Methods). The homology vector 752-22.1 is further improved by placing the lacZ gene under the control of the synthetic LP1 promoter. The LP1 promoter results in higher levels of lacZ expression compared to the SPV O1L promoter

Example 25

S-SPV-041:

S-SPV-041 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* ß-galactosidase (lacZ) and the gene for equine herpesvirus type 1 glycoprotein B (gB) were inserted into the 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of nucleotides 1679 to 2452, SEQ ID NO: 189). The lacZ gene is under the control of the swinepox O1L promoter, and the EHV-1 gB gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-041 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 752-29.33 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-041. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-041 is useful as a vaccine in horses against EHV-1 infection and is useful for expression of EHV-1 glycoprotein B.

S-SPV-045:

S-SPV-045 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* ß-galactosidase (lacZ) and the gene for infectious bovine rhinotracheitis virus glycoprotein E (gE) were inserted into the 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of nucleotides 1679 to 2452, SEQ ID NO: 189). The lacZ gene is under the control of the swinepox O1L promoter, and the IBRV gE gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-045 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 746-94.1 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-045. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-045 is useful for expression of IBRV glycoprotein E.

S-SPV-049:

S-SPV-049 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* ß-galactosidase (lacZ) and the gene for bovine viral diarrhea virus glycoprotein 48 (gp48) were inserted into the 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of nucleotides 1679 to 2452, SEQ ID NO: 189). The lacZ gene is under the control of the swinepox O1L promoter, and the BVDV gp48 gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-049 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 771-55.11 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-049. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-049 is useful as a vaccine in cattle against BVDV infection and is useful for expression of BVDV glycoprotein 48.

S-SPV-050:

S-SPV-050 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* ß-galactosidase (lacZ) and the gene for the bovine viral diarrhea virus glycoprotein 53 (gp53) were inserted into the 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF;

Deletion of nucleotides 1679 to 2452, SEQ ID NO: 189). The lacZ gene is under the control of the swinepox O1L promoter, and the IBRV gE gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-050 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 767-67.3 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-050. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-050 is useful as a vaccine in cattle against BVDV infection and is useful for expression of BVDV glycoprotein 53.

Example 26

Recombinant swinepox virus, S-SPV-042 or S-SPV-043, expressing chicken interferon (cIFN) or chicken myelomonocytic growth factor (cMGF), respectively, are useful to enhance the immune response when added to vaccines against diseases of poultry. Chicken myelomonocytic growth factor (cMGF) is homologous to mammalian interleukin-6 protein, and chicken interferon (cIFN) is homologous to mammalian interferon. When used in combination with vaccines against specific avian diseases, S-SPV-042 and S-SPV-043 provide enhanced

mucosal, humoral, or cell mediated immunity against avian disease-causing viruses including, but not limited to, Marek's disease virus, Newcastle disease virus, infectious laryngotracheitis virus, infectious bronchitis virus, infectious bursal disease virus.

Example 26A

S-SPV-042:

S-SPV-042 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* ß-galactosidase (lacZ) and the gene for chicken interferon (cIFN) were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the cIFN gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-042 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 751-07.A1 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-042. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-042 has interferon activity in cell culture. Addition of S-SPV-042 conditioned media to chicken embryo fibroblast (CEF) cell culture inhibits infection of the CEF cells by vesicular stomatitis virus or by herpesvirus of turkeys. S-SPV-042 is useful to enhance the immune response when added to vaccines against diseases of poultry.

Example 26B

S-SPV-043:

S-SPV-043 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* ß-galactosidase (lacZ) and the gene for chicken myelomonocytic growth factor (cMGF) were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the cMGF gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-043 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 751-56.A1 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-043. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-043 is useful to enhance the immune response when added to vaccines against diseases of poultry.

Example 27

Insertion into a non-essential site in the 2.0 kb HindIII to BglII region of the swinepox virus HindIII M fragment.

A 2.0 kb HindIII to BglII region of the swinepox virus HindIII M fragment is useful for the insertion of foreign DNA into SPV. The foreign DNA is inserted into a unique BglII restriction site in the region Nucleotide 540 of SEQ ID NOs: 195). A plasmid containing a foreign DNA insert is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV to generate an SPV containing the foreign DNA. For this procedure to be successful, it is important that the insertion site be in a region non-essential to the replication of the SPV and that the site be flanked with swinepox virus DNA appropriate for mediating homologous recombination between virus and plasmid DNAs. The unique BglII restriction site in the 2.0 kb HindIII to BglII region of the swinepox virus HindIII M fragment is located within the coding region of the SPV I4L open reading frame. The I4L ORF has sequence similarity to the vaccinia virus and smallpox virus ribonucleotide reductase (large subunit) gene (56-58). The ribonucleotide reductase (large subunit) gene is non-essential for DNA replication of vaccinia virus and is an appropriate insertion site in swinepox virus.

Example 28

S-SPV-047

S-SPV-047 is a swinepox virus that expresses two foreign genes. The gene for E. coli ß-galactosidase (lacZ) and the gene for pseudorabies virus gB (gII) were inserted into a unique HindIII site (HindIII linker inserted into the BglII restriction endonuclease site within the 2.0 kb BglII to HindIII subfragment of the HindIII M fragment.) The BglII insertion site is within the SPV I4L open reading frame which has significant homology to the vaccinia virus ribonucleoside-diphosphate reductase gene. The lacZ gene is under the control of the synthetic late promoter (LP1), and the PRV gB (gII) gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-047 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 779-94.31 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-047. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-047 was assayed for expression of PRV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-

PRV serum was shown to react specifically with S-SPV-047 plaques and not with S-SPV-001 negative control plaques. All S-SPV-047 observed plaques reacted with the swine anti-PRV serum indicating that the virus was stably expressing the PRV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

To confirm the expression of the PRV gB gene product, cells were infected with S-SPV-047 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal swine anti-PRV serum was used to detect expression of PRV specific proteins. The cell lysate and supernatants from S-SPV-047 infected cells exhibited bands corresponding to 120 kD, 67 kD and 58 kD, which are the expected size of the PRV glycoprotein B.

SPV recombinant-expressed PRV gB has been shown to elicit a significant immune response in swine (37, 38; See example 8). Furthermore, PRV gB is expressed in recombinant SPV, significant protection from challenge with virulent PRV is obtained. (See Examples 6 and 8) Therefore S-SPV-047 is valuable as a vaccine to protect swine against PRV disease. Since the PRV vaccines described here do not express PRV gX or gI, they would be compatible with current PRV diagnostic tests (gX HerdChek®, gI HerdChek® and ClinEase®) which are utilized to distinguish vaccinated animals from infected animals.

<u>S-SPV-052</u>

S-SPV-052 is a swinepox virus that expresses three foreign genes. The gene for E. coli ß-galactosidase (lacZ) and the gene for pseudorabies virus gB (gII) were inserted into the unique HindIII restriction site (HindIII linkers inserted into a unique NdeI site in the SPV O1L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104;) of the SPV HindIII M fragment has been deleted). The gene for PRV gD (g50) was inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The lacZ gene is under the control of the synthetic late promoter (LP1), the PRV gB (gII) gene is under the control of the synthetic late/early promoter (LP2EP2), and the PRV gD (g50) gene is under the control of the synthetic early/late promoter (EP1LP2).

S-SPV-052 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 789-41.7 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 052. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-052 was assayed for expression of PRV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-PRV serum was shown to react specifically with S-SPV-

052 plaques and not with S-SPV-001 negative control plaques. All S-SPV-052 observed plaques reacted with the swine anti-PRV serum indicating that the virus was stably expressing the PRV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

To confirm the expression of the PRV gB and gD gene products, cells were infected with S-SPV-052 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal swine anti-PRV serum was used to detect expression of PRV specific proteins. The cell lysate and supernatants from S-SPV-052 infected cells exhibited bands corresponding to 120 kD, 67 kD and 58 kD, which are the expected size of the PRV glycoprotein B; and a 48 kD which is the expected size of the PRV glycoprotein D.

SPV recombinant-expressed PRV gB and gD has been shown to elicit a significant immune response in swine (37, 38; See example 8). Furthermore, PRV gB and gD are expressed in recombinant SPV, significant protection from challenge with virulent PRV is obtained. (See Examples 6 and 8) Therefore S-SPV-052 is valuable as a vaccine to protect swine against PRV disease. Since the PRV vaccines described here do not express PRV gX or gI, they would be compatible with current PRV diagnostic tests (gX HerdChek®, gI HerdChek® and ClinEase®) which are utilized to distinguish vaccinated animals from infected animals.

<u>S-SPV-053</u>

S-SPV-053 is a swinepox virus that expresses three foreign genes. The gene for E. coli ß-galactosidase (lacZ) and the gene for pseudorabies virus gB (gII) were inserted into the unique HindIII restriction site (HindIII linkers inserted into a unique NdeI site in the SPV O1L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104;) of the SPV HindIII M fragment has been deleted). The gene for PRV gC (gIII) was inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV O1L open reading frame). The lacZ gene is under the control of the synthetic late promoter (LP1), the PRV gB (gII) gene is under the control of the synthetic late/early promoter (LP2EP2), and the PRV gC (gIII) gene is under the control of the synthetic early/late promoter (EP1LP2).

S-SPV-053 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 789-41.27 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 053. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-053 was assayed for expression of PRV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-PRV serum was shown to react specifically with S-SPV-

053 plaques and not with S-SPV-001 negative control plaques. All S-SPV-053 observed plaques reacted with the swine anti-PRV serum indicating that the virus was stably expressing the PRV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

To confirm the expression of the PRV gB and gC gene products, cells were infected with S-SPV-053 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal swine anti-PRV serum was used to detect expression of PRV specific proteins. The cell lysate and supernatants from S-SPV-053 infected cells exhibited bands corresponding to 120 kD, 67 kD and 58 kD, which are the expected size of the PRV glycoprotein B; and a 92 kD which is the expected size of the PRV glycoprotein C.

SPV recombinant-expressed PRV gB and gC has been shown to elicit a significant immune response in swine (37, 38; See example 8). Furthermore, PRV gB and gC are expressed in recombinant SPV, significant protection from challenge with virulent PRV is obtained. (See Examples 6 and 8) Therefore S-SPV-053 is valuable as a vaccine to protect swine against PRV disease. Since the PRV vaccines described here do not express PRV gX or gI, they would be compatible with current PRV diagnostic tests (gX HerdChek®, gI HerdChek® and ClinEase®) which are utilized to distinguish vaccinated animals from infected animals.

<u>S-SPV-054</u>

S-SPV-054 is a swinepox virus that expresses three foreign genes. The gene for E. coli ß-galactosidase (lacZ) and the gene for pseudorabies virus gC (gIII) were inserted into the unique HindIII restriction site (HindIII linkers inserted into a unique NdeI site in the SPV O1L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104;) of the SPV HindIII M fragment has been deleted). The gene for PRV gD (g50) was inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The lacZ gene is under the control of the synthetic late promoter (LP1), the PRV gC (gIII) gene is under the control of the synthetic early/late promoter (EP1LP2), and the PRV gD (g50) gene is under the control of the synthetic early/late promoter (EP1LP2).

S-SPV-054 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 789-41.47 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 054. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-054 was assayed for expression of PRV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-

PRV serum was shown to react specifically with S-SPV-054 plaques and not with S-SPV-001 negative control plaques. All S-SPV-054 observed plaques reacted with the swine anti-PRV serum indicating that the virus was stably expressing the PRV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

To confirm the expression of the PRV gC and gD gene products, cells were infected with S-SPV-054 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal swine anti-PRV serum was used to detect expression of PRV specific proteins. The cell lysate and supernatants from S-SPV-054 infected cells exhibited a band corresponding to 92 kD which is the expected size of the PRV glycoprotein C and a 48 kD which is the expected size of the PRV glycoprotein D.

SPV recombinant-expressed PRV gC and gD has been shown to elicit a significant immune response in swine (37, 38; See example 8). Furthermore, PRV gC and gD are expressed in recombinant SPV, significant protection from challenge with virulent PRV is obtained. (See Examples 6 and 8) Therefore S-SPV-054 is valuable as a vaccine to protect swine against PRV disease. Since the PRV vaccines described here do not express PRV gX or gI, they would be compatible with current PRV diagnostic tests (gX HerdChek®, gI HerdChek® and ClinEase®) which are utilized to distinguish vaccinated animals from infected animals.

S-SPV-055

S-SPV-055 is a swinepox virus that expresses four foreign genes. The gene for E. coli ß-galactosidase (lacZ) and the gene for pseudorabies virus gB (gII) were inserted into the unique HindIII restriction site (HindIII linkers inserted into a unique NdeI site in the SPV 01L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104;) of the SPV HindIII M fragment has been deleted). The gene for PRV gD (g50) and PRV gC (gIII) were inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The lacZ gene is under the control of the synthetic late promoter (LP1), the PRV gB (gII) gene is under the control of the synthetic late/early promoter (LP2EP2), the PRV gD (g50) gene is under the control of the synthetic late/early promoter (LP2EP2), and the PRV gC (gIII) gene is under the control of the synthetic early/late promoter (EP1LP2).

S-SPV-055 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 789-41.73 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 055. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-055 was assayed for expression of PRV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE

EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-PRV serum was shown to react specifically with S-SPV-055 plaques and not with S-SPV-001 negative control plaques. All S-SPV-055 observed plaques reacted with the swine anti-PRV serum indicating that the virus was stably expressing the PRV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

To confirm the expression of the PRV gB, gC and gD gene products, cells were infected with S-SPV-055 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal swine anti-PRV serum was used to detect expression of PRV specific proteins. The cell lysate and supernatants from S-SPV-055 infected cells exhibited a bands corresponding to 120 kD, 67 kD, and 58 kD which is the expected size of the PRV glycoprotein B; a 92 kD which is the expected size of the PRV glycoprotein C; and a 48 kD which is the expected size of the PRV glycoprotein D

SPV recombinant-expressed PRV gB, gC and gD has been shown to elicit a significant immune response in swine (37, 38; See example 8). Furthermore, PRV gB, gC and gD are expressed in recombinant SPV, significant protection from challenge with virulent PRV is obtained. (See Examples 6 and 8) Therefore S-SPV-055 is valuable as a vaccine to protect swine against PRV disease. Since the PRV vaccines described here do not express PRV gX or gI, they would be compatible with current PRV diagnostic tests (gX HerdChek®, gI

HerdChek® and ClinEase®) which are utilized to distinguish vaccinated animals from infected animals.

Example 29

S-SPV-059

S-SPV-059 is a swinepox virus that expresses one foreign gene. The gene for E. coli B-glucuronidase (uidA) was inserted into the unique EcoRI restriction site in the SPV B18R open reading frame within the SPV HindIII K genomic fragment. The uidA gene is under the control of the synthetic late/early promoter (LP2EP2). Partial sequence of the SPV 3.2 kb region of the SPV 6.5 kb HindIII K fragment indicates three potential open reading frames. The SPV B18R ORF shows sequences homology to the vaccinia virus B18R gene, 77.2K protein from rabbit fibroma virus, vaccinia virus C19L/B25R ORF and an ankyrin repeat region from a human brain variant. The B18R gene codes for a soluble interferon receptor with high affinity and broad specificity. The SPV B4R open reading frame shows sequence homology to the T5 protein of rabbit firbroma virus.

S-SPV-059 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 796-50.31 and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. Homology vector 796-50.31 was generated by insertion of a blunt ended NotI fragment containing the LP2EP2 promoter uidA cassette from plasmid 551-47.23 (see Materials and Methods) into a unique EcoRI site (blunt ended) in the SPV 6.5 kb HindIII K fragment, (Figure 29B). The transfection stock was screened by the SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES. The final result of blue plaque purification was the recombinant virus designated S-SPV-059. This virus

was assayed for B-glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-059 has been purified and expresses the foreign gene, E. coli uida, indicating that the EcoRI site within the 6.5 kb HindIII K fragment is a stable insertion site for foreign genes. Recombinant swinepox virus utilizing this insertion site is useful for expression of foreign antigen genes, as a vaccine against disease or as an expression vector to raise antibodies to the expressed foreign gene.

S-SPV-060

S-SPV-060 is a swinepox virus that expresses one foreign gene. The gene for E. coli B-glucuronidase (uidA) was inserted into the unique EcoRV restriction site within the SPV HindIII N genomic fragment. The uidA gene is under the control of the synthetic late/early promoter (LP2EP2). Partial sequence of the SPV 3.2 kb HindIII N fragment (SEQ ID NO.) indicates two potential open reading frames. The SPV I7l ORF shows sequences homology to protein I7 of vaccinia virus. The SPV I4L open reading frame shows sequence homology to the ribonucleoside diphosphate reductase gene of vaccinia virus. Two potential open reading frames I5L and I6L, between I4L adn I7L ORF are of unknown function.

S-SPV-060 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 796-71.31 and virus S-SPV-001 in the HOMOLOGOUS

RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. Homology vector 796-71.31 was generated by insertion of a blunt ended NotI fragment containing the LP2EP2 promoter uidA cassette from plasmid 551-47.23 (see Materials and Methods) into a unique EcoRV site in the SPV 3.2 kb HindIII N fragment (Figure 11A). The transfection stock was screened by the SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES. The final result of blue plaque purification is the recombinant virus designated S-SPV-060. This virus is assayed for ß-glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign gene.

S-SPV-060 is purified and expresses the foreign gene, E. coli uidA, indicating that the EcoRI site within the 3.2 kb HindIII N fragment is a stable insertion site for foreign genes. Recombinant swinepox virus utilizing this insertion site is useful for expression of foreign antigen genes, as a vaccine against disease or as an expression vector to raise antibodies to the expressed foreign gene.

S-SPV-061

S-SPV-061 is a swinepox virus that expresses one foreign gene. The gene for E. coli ß-glucuronidase (uidA) was inserted into the unique SnaBI restriction site within the SPV HindIII N genomic fragment. The uidA gene is under the control of the synthetic late/early promoter (LP2EP2). Partial sequence of the SPV 3.2 kb HindIII N fragment indicates two potential open reading frames. The SPV I7L ORF shows sequence

homology to protein 17 of vaccinia virus. The SPV I4L open reading frame shows sequence homology to the ribonucleoside diphosphate reductase gene of vaccinia virus. Two potential open reading frames I5L and I6L, between I4L ORF and I7L ORF are of unknown function.

S-SPV-061 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 796-71.41 and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. Homology vector 796-71.41 was generated by insertion of a blunt ended NotI fragment containing the LP2EP2 promoter uidA cassette from plasmid 551-47.23 (see Materials and Methods) into a unique SnaBI site in the SPV 3.2 kb HindIII N fragment. The transfection stock was screened by the SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES. The final result of blue plaque purification is the recombinant virus designated S-SPV-061. This virus is assayed for ß-glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign gene.

S-SPV-061 is purified and expresses the foreign gene, E. coli uidA, indicating that the SnaBI site within the 3.2 kb HindIII N fragment is a stable insertion site for foreign genes. Recombinant swinepox virus utilizing this insertion site is useful for expression of foreign antigen genes, as a vaccine against disease or as an expression vector to raise antibodies to the expressed foreign gene.

S-SPV-062

S-SPV-062 is a swinepox virus that expresses one foreign gene. The gene for E. coli ß-glucuronidase (uidA) was inserted into the unique BglII restriction site within the SPV HindIII N genomic fragment (Figure 11A). The uidA gene is under the control of the synthetic late/early promoter (LP2EP2). Partial sequence of the SPV 3.2 kb HindIII N fragment indicates two potential open reading frames. The SPV I7L ORF shows sequence homology to protein 17 of vaccinia virus. The SPV I4L open reading frame shows sequence homology to the ribonucleoside diphosphate reductase gene of vaccinia virus. Two potential open reading frames I5L and I6L, between I4L ORF and I7L ORF are of unknown function.

S-SPV-062 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 796-71.51 and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. Homology vector 796-71.51 was generated by insertion of a blunt ended NotI fragment containing the LP2EP2 promoter uidA cassette from plasmid 551-47.23 (see Materials and Methods) into a unique BglII site in the SPV 3.2 kb HindIII N fragment. The transfection stock was screened by the SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES. The final result of blue plaque purification is the recombinant virus designated S-SPV-062. This virus is assayed for ß-glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign gene.

S-SPV-062 is purified and expresses the foreign gene, E. coli uidA, indicating that the BglII site within the

3.2 kb HindIII N fragment is a stable insertion site for foreign genes. Recombinant swinepox virus utilizing this insertion site is useful for expression of foreign antigen genes, as a vaccine against disease or as an expression vector to raise antibodies to the expressed foreign gene.

Example 30:

Recombinant swinepox virus expressing E coli ß-galactosidase (lacZ) under the control of a synthetic early or synthetic late pox promoter.

Three recombinant swinepox viruses, S-SPV-056, S-SPV-057, and S-SPV-058 expressing E coli ß-galactosidase (lacZ) under the control of a synthetic pox promoter, LP1, LP2, and EP1, respectively, have been constructed.

S-SPV-056 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 791-63.19 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). S-SPV-057 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 791-63.41 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). S-SPV-058 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 796-18.9 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by

the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification were the recombinant viruses designated S-SPV-056, S-SPV-057 and S-SPV-058. The viruses were assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

Recombinant swinepox virus expresses a foreign gene such as E. coli ß-galactosidase in a human cell line but does not replicate in the human cell line. To optimize expression of the foreign gene, S-SPV-056, S-SPV-057 and S-SPV-058 are used to compare optimal expression levels of E. coli ß-galactosidase under the control of early or late synthetic pox viral promoters. The human cell lines in which expression of recombinant swinepox virus has been detected include, but are not limited to 143B (osteosarcoma), A431 (epidermoid carcinoma), A549 (lung carcinoma), Capan-1 (liver carcinoma), CF500 (foreskin fibroblasts), Chang Liver (liver), Detroit (down's foreskin fibroblasts), HEL-199 (embryonic lung), HeLa (cervical carcinoma), HEp-2 (epidermal larynx carcinoma), HISM (intestinal smooth muscle), HNK (neonatal kidney), MRC-5 (embryonic lung), NCI-H292 (pulmonary mucoepidermoid carcinoma), OVCAR-3 (ovarian carcinoma), RD (rhabdosarcoma), THP (monocyte leukemia), WIL2-NS (B lymphocyte line, non-secreting), WISH (amnion).

Example 31:

S-SPV-051

S-SPV-051 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli ß-galactosidase (lacZ) and the gene for the bovine viral diarrhea virus glycoprotein 53 (g53) were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the BVDV g53 gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-051 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 783-39.2 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 051. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-051 was assayed for expression of BVDV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. A mouse monoclonal antibody to BVDV g53 was shown to react specifically with S-SPV-051 plaques and not with S-SPV-001 negative control plaques. All S-SPV-051 observed plaques reacted with the monoclonal antibody to BVDV g53 indicating that the virus was stably expressing the BVDV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4

cells would be a suitable substrate for the production of SPV recombinant vaccines.

To confirm the expression of the BVDV g53 gene product, cells were infected with S-SPV-051 and samples of infected cell lysates and culture supern atants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A mouse monoclonal antibody to BVDV g53 was used to detect expression of BVDV specific proteins. The cell lysate and supernatant from S-SPV-051 infected cells exhibited bands at 53 kd and higher indicating glycosylated and unglycosylated forms of the BVDV g53 protein.

S-SPV-051 is useful as a vaccine in cattle against BVDV infection and is useful for expression of BVDV glycoprotein 53.

Example 32:

S-SPV-044:

S-SPV-044 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli ß-galactosidase (lacZ) and the gene for the infectious bursal disease virus (IBDV) polymerase protein were inserted into the 617-48.1 ORF (a unique NotI site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the IBDV polymerase gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-044 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 749-75.78 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR

GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-044. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-044 is useful for expression of IBDV polymerase protein. S-SPV-044 is useful in an *in vitro* approach to a recombinant IBDV attenuated vaccine. RNA strands from the attenuated IBDV strain are synthesized in a bacterial expression system using T3 or T7 promoters (pBlueScript plasmid; Stratagene, Inc.) to synthesize double stranded short and long segments of the IBDV genome. The IBDV double stranded RNA segments and S-SPV-044 are transfected into CEF cells. The swinepox virus expresses the IBDV polymerase but does not replicate in CEF cells. The IBDV polymerase produced from S-SPV-044 synthesizes infectious attenuated IBDV virus from the double stranded RNA genomic templates. The resulting attenuated IBDV virus is useful as a vaccine against infectious bursal disease in chickens.

Example 33:

S-SPV-046:

S-SPV-046 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli ß-galactosidase (lacZ) and the gene for the feline immunodeficiency virus (FIV) gag protease (gag) were inserted into the 738-94.4 ORF (a 773 base pair deletion of the SPV O1L

ORF; Deletion of nucleotides 1669 to 2452, SEQ ID NO: 189). The lacZ gene is under the control of the swinepox O1L promoter, and the FIV gag gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-046 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 761-75.B18 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 046. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

To confirm the expression of the FIV gag gene product, cells were infected with S-SPV-046 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. Feline anti-FIV (PPR strain) sera was used to detect expression of FIV specific proteins. The cell lysate and supernatant from S-SPV-046 infected cells exhibited bands at 26 kd and 17 kd which are the expected sizes of the processed form of the FIV gag protein. The recombinant swinepox virus expressed FIV gag protein is processed properly and secreted into the culture media.

S-SPV-048

S-SPV-048 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli ß-galactosidase (lacZ) and the gene for feline immunodeficiency virus (FIV) envelope (env) were inserted into the SPV 617 48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the FIV env gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-048 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 781-84.C11 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 048. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-046 and S-SPV-048 are useful alone or in combination as a vaccine in cats against FIV infection and are useful for expression of the FIV env and gag proteins. A recombinant swinepox virus expressing both the FIV env and gag proteins is useful as a vaccine in cats against FIV infection.

Recombinant swinepox virus expressing human respiratory synctial virus F and G proteins is useful as a vaccine against the human disease.

Example 34

In Vitro Properties of Chicken IFN Expressed in Recombinant Pox viruses.

Growth properties of recombinant viruses in cell culture. Gowth properties of recombinant S-SPV-042 were not effected in embryonic swine kidney cells (ESK-4) compared to wild-type swinpox virus.

Western blot analysis was performed on supernatants from cells infected with SPV/cIFN recombinant virus. Rabbit and mouse antisera were raised against cIFN from concentrated SPV/cIFN infected supernatants and pre-cleared against ESK-4 cells infected with wild-type SPV in preparation for western analysis. Rabbit and mouse anti-cIFN antisera were reacted with denatured proteins on nitrocellulose from recombinant SPV/cIFN and SPV wild type virus infected supernatants. A reactive band with an estimated molecular weight size range of 17-20 kilodaltons was present in the SPV/cIFN lanes, and absent in the SPV wild type control lanes.

Effect of cIFN expressed in supernatants from SPV/cIFN (S-SPV-042), FPV/cIFN, and FPV/cIFN/NDV infected cells on the growth of Vesicular Stomatis Virus.

Virion cleared supernatants from SPV/cIFN, FPV/cIFN and FPV/cIFN/NDV infected cells were tested for the presence of viral inhibitory activity, results shown in Table 1. Briefly, CEF cells were incubated with serially diluted viral supernatants. Subsequently, 40,000 plaque forming units (pfu)/well of vesicular stomatitis virus (VSV) were added and 48 hours later, wells were scored for the presence of VSV cytopathic effect (CPE). Recombinant viral supernatants

containing cIFN were shown to inhibit VSV induced CPE, whereas, control viral supernatants did not. VSV induced cytopathic effect could be reversed in the presence of rabbit anti-cIFN sera.

Table 1.

Recombinant Viral Supernatants.	cIFN Activity (units/ml).[a]
SPV/IFN	2,500 000
SPV	<100
FPV/IFN	250,000
FPV/cIFN/NDV	250,000
FPV	<100

[a]. One unit of cIFN activity is defined as the dilution of pox virus supernatant at which 100% VSV CPE was inhibited.

<u>Effect of cIFN expressed from supernatants of SPV/cIFN infected cells on herpes virus of turkeys.</u>

Supernatant containing recombinant cIFN from ESK-4 cells infected with SPV/cIFN virus, was tested for its ability to inhibit the growth of herpes virus of turkeys (HVT) in CEF cells, results shown in Table 2. Briefly, serially diluted supernatants were incubated with CEF cells, and then subsequently infected with 100 pfu/well of wild-type HVT. Plaques were counted in all wells after 48 hours. It was shown that 10-100 units of cIFN activity inhibited plaque formation of HVT(100 pfu/well). Supernatants from wild type SPV did not inhibit HVT plaque formation.

Table 2.

SPV/cIFN Supernatant (units/ml[a])	Number of HVT plaques
0	99
1000	0
100	0
10	45

a. One unit of cIFN activity is defined as the dilution of pox virus supernatant at which 100% VSV CPE was inhibited.

Induction of NO by chicken macrophages after treatment with cIFN expressed in supernatants from SPV/cIFN infected cells.

HD 11 cells or bone marrow adherent cells were incubated with 1000unit/ml of cIFN from SPV/cIFN supernatants, lipopolysaccharide (LPS) (6ng/ml) or with both cIFN and LPS, results shown in Table 3. After 24 hours, supernatant fluids were collected and nitrite levels were measured. These data demonstrate that cIFN expressed from SPV/cIFN supernatants has the ability to activate chicken macrophages in the presence of LPS.

Table 3.

	Nitrite (micro/mol) levels following stimulation with :		
Cell source	LPS	SPV/cIFN	LPS + SPV/cIFN
HD11	10.76	6.4	35.29
BMAC	13.1	5.8	35.10

Conclusions:

1. Recombinant swinepox viruses express biologically active chicken interferon into the supernatants of infected cells, as measured by protection of CEF cells from VSV infection.

2. Chicken interferon expressed in supernatants from recombinant SPV/cIFN infected cells has been shown to protect CEF cells against infection with HVT in a dose dependent manner.

3. Chicken interferon expressed from SPV/cIFN acted synergistically with LPS to activate chicken macrophages as detected by nitric oxide induction.

4. The foregoing data indicate that recombinant swinepox viruses expressing chicken IFN may have beneficial applications as immune modulating agents *in vitro, in vivo* and *in ovo*.

Example 35

As an alternative to the construction of a IBD vaccine using a viral vectored delivery system and/or subunit approaches, IBD virus RNA is directly manipulated re-constructing the virus using full length RNA derived from cDNA clones representing both the large (segment A) and small (segment B) double-stranded RNA subunits. Generation of IBD virus is this manner offers several advantages over the first two approaches. First, if IBD virus is re-generated using RNA templates, one is able to manipulate the cloned cDNA copies of the viral genome prior to transcription (generation of RNA). Using this approach, it is possible to either attenuate a virulent IBD strain or replace the VP2 variable region of the attenuated vaccine backbone with that of virulent strains. In doing so, the present invention provides protection against the virulent IBDV strain

while providing the safety and efficacy of the vaccine strain. Furthermore, using this approach, the present invention constructs and tests temperature sensitive IBD viruses generated using the RNA polymerase derived from the related birnavirus infectious pancreatic necrosis virus (IPNV) and the polyprotein derived from IBDV. The IPNV polymerase has optimum activity at a temperature lower than that of IBDV. If the IPNV polymerase recognizes the regulatory signals present on IBDV, the hybrid virus is expected to be attenuated at the elevated temperature present in chickens. Alternatively, it is possible to construct and test IBD viruses generated using the RNA polymerase derived from IBDV serotype 2 viruse and the polyprotein derived from IBDVserotype 1 virus..

cDNA clones representing the complete genome of IBDV (double stranded RNA segments A and B) is constructed, initially using the BursaVac vaccine strain (Sterwin Labs). Once cDNA clones representing full length copies of segment A and B are constructed, template RNA is prepared. Since IBDV exists as a bisegmented double-stranded RNA virus, both the sense and anti-sense RNA strands of each segment are produced using the pBlueScript plasmid; Stratagene, Inc.). These vectors utilize the highly specific phage promoters SP6 or T7 to produce substrate amounts of RNA *in vitro*. A unique restriction endonuclease site is engineered into the 3' PCR primer to linearize the DNA for the generation of run-off transcripts during transcription.

The purified RNA transcripts (4 strands) are transfected into chick embryo fibroblasts (CEF) cells to determine whether the RNA is infectious. If IBD virus is generated, as determined by black plaque assays using IBDV specific Mabs, no further manipulations are required and engineering of the

vaccine strain can commence. The advantage of this method is that engineered IBD viruses generated in this manner will be pure and require little/no purification, greatly decreasing the time required to generate new vaccines. If negative results are obtained using the purified RNA's, functional viral RNA polymerase is required by use of a helper virus. Birnaviruses replicate their nucleic acid by a strand displacement (semi-conservative) mechanism, with the RNA polymerase binding to the ends of the double-stranded RNA molecules forming circularized ring structures (Muller & Nitschke, Virology 159, 174-177, 1987). RNA polymerase open reading frame of about 878 amino acids in swinepox virus is expressed and this recombinant virus (S-SPV-044) is used to provide functional IBDV RNA polymerase *in trans*. Swinpox virus expressed immunologically recognizable foreign antigens in avian cells (CEF cells), where there are no signs of productive replication of the viral vector. In the present invention the IBDV polymerase protein is expressed in the same cells as the transfected RNA using the swinepox vector without contaminating the cells with SPV replication.

With the demonstration that IBD virus is generated *in vitro* using genomic RNA, an improved live attenuated virus vaccines against infectious bursal disease is developed. Using recombinant DNA technology along with the newly defined system of generating IBD virus, specific deletions within the viral genome, facilitating the construction of attenuated viruses are made. Using this technology, the region of IBDV responsible for virulence and generate attenuated, immunogenic IBDV vaccines are identified. The present invention provides a virulent IBD strain or replacement of the VP2 variable region of the attenuated vaccine backbone with that of a virulent strain, thus

protecting against the virulent strain while providing the safety and efficacy of the vaccine strain.

Example 36

Effects of Rabbit anti-chicken interferon (cIFN) antibody on the growth of Herpes Virus of Turkeys.

Supernatants from SPV/cIFN (SPV 042) infected ESK-4 cells were harvested 48 hours after infection and then concentrated 5-10 times, by Centricon 10 columns (Amicon). One ml of concentrated supernatant was injected into a rabbit 3 times, at 3 week intervals, and then bled. This rabbit antisera was then used in culture to study the effect of interferon on the growth of HVT. It was shown that anti-cIFN reverses the block to HVT (1:200) and VSV(1:80) growth induced by the addition of cIFN in plaque assays. Furthermore, it was shown that the addition of anti-cIFN (1:100) in the media of CEFs transiently transfected with sub-plaqueing levels of HVT viral DNA, enhances the formation of HVT plaques (200 plaques/well). CEFs transfected with HVT DNA in the absence of anti-cIFN did not yield plaques.

HVT is highly susceptible to interferon produced from CEFs and that when cIFN is blocked, HVT growth is enhanced.

Applications include: (1) Use antibody to cIFN as an additive to increase HVT titers in vaccine stocks; (2) Use antibody to cIFN as an additive to facilitate the formation of new recombinant HVT viruses via cosmid reconstructions.

S-SPV-063

S-SPV-063 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli ß-galactosidase (lacZ) and the gene for swine influenza virus (SIV) NP (H1N1) were inserted into the SPV 617 48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the SIV NP gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-063 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 807-41.3 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 063. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-063 was assayed for expression of SIV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-SIV serum or a polyclonal goat anti-NP serum was shown to react specifically with S-SPV-063 plaques and not with S-SPV-001 negative control plaques. All S-SPV-063 observed plaques reacted with the swine anti-SIV serum or goat anti-NP serum indicating that the virus was stably expressing the SIV foreign gene. The assays

described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

To confirm the expression of the SIV NP gene products, cells were infected with S-SPV-063 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis, The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal swine anti-SIV serum or a polyclonal goat anti-NP serum was used to detect expression of SIV specific proteins. The cell lysate and supernatant from S-SPV-063 infected cells exhibited bands corresponding to 56 kd, which is the expected size of the SIV NP protein.

S-SPV-063 is useful as a vaccine in swine against SIV infection and is useful for expression of SIV NP. S-SPV-063 is useful as a vaccine in combination with S-SPV-066 which expresses NA and S-SPV-065 which expresses SIV HA.

<u>S-SPV-064</u>

S-SPV-064 is a swinepox virus that expresses one foreign gene. The gene for E. coli ß-glucuronidase (uidA) was inserted into the unique XhoI restriction site within the 6.9 kb SPV HindIII J genomic fragment. The uidA gene is under the control of the synthetic late/early promoter (LP2EP2). The HindIII J genomic fragment contains part of the A50R ORF (aa 227 to 552). The unique XhoI site is not within the A50R ORF. The XhoI site is 25 kb from the 3'end of the swinepox virus genome (62).

S-SPV-064 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 807-42.28 and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. Homology vector 807-42.28 was generated by insertion of a NotI fragment containing the LP2EP2 promoter uidA gene cassette from plasmid 551-47.23 (see Materials and Methods) into a NotI site (unique XhoI site converted to NotI by a DNA linker) in the SPV 6.9 kb HindIII J fragment. The transfection stock was screened by the SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES. The final result of blue plaque purification is the recombinant virus designated S-SPV-064. This virus is assayed for ß-glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign gene.

S-SPV-064 is purified and expresses the foreign gene, E. coli uidA, indicating that the XhoI site within the 6.9 kb HindIII J fragment is a site non-essential for virus growth and a stable insertion site for foreign genes. Recombinant swinepox virus utilizing this insertion site is useful for expression of foreign antigen genes, as a vaccine against disease or as an expression vector to raise antibodies to the expressed foreign gene.

S-SPV-065

S-SPV-065 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli ß-galactosidase (lacZ) and the gene for swine influenza virus (SIV) HA (H1N1) were inserted into the SPV 617 48.1 ORF (a

unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the SIV HA gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-065 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 807-84.8 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 065. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene. S-SPV-065 was assayed for expression of SIV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-SIV serum or a Polyclonal goat anti-HA serum was shown to react specifically with S-SPV-065 plaques and not with S-SPV-001 negative control plaques. All S-SPV-065 observed plaques reacted with the swine anti-SIV serum or the SIV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

To confirm the expression of the SIV NP gene products, cells were infected with S-SPV-065 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis.

The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A Polyclonal swine anti-SIV serum or a Polyclonal goat anti-HA serum was used to detect expression SIV specific proteins. The cell lysate and supernatant from S-SPV-065 infected cells exhibited bands corresponding to 64 kd, which is the expected size of the SIV-HA protein.

S-SPV-065 is useful as a vaccine in swine against SIV infection and is useful for expression of SIV HA. S-SPV-065 is useful as a vaccine in combination with S-SPV-066 which expresses NA and S-SPV-063 which expresses SIV NP.

S-SPV-066

S-SPV-066 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli ß-galactosidase (lacZ) and the gene for swine influenza virus (SIV) NA (H1N1) were inserted into the SPV 617--48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the SIV NA gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-066 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 807-84.35 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 066. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue

plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

To confirm the expression of the SIV NA gene products, cells were infected with S-SPV-066 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A Polyclonal swine anti-SIV serum or a Polyclonal goat anti-NA serum was used to detect expression of SIV specific proteins. The cell lysate and supernatant from S-SPV-066 infected cells exhibited bands corresponding to 64 kd, which is the expected size of the SIV HA protein.

S-SPV-066 is useful as a vaccine in swine against SIV infection and is useful for expression of SIV-NA. S-SPV-066 is useful as a vaccine in combination with S-SPV-065 which expresses HA and S-SPV-063 which expresses SIV NP.

<u>S-SPV-071</u>

S-SPV-071 is a swinepox virus that expresses at least four foreign genes. The gene for E. coli ß-galactosidase (lacZ) and the genes for swine influenza virus (SIV) HA (H1N1) and NA (H1N1) were inserted into the SPV 617 48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the SIV HA, and NA genes are under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-071 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 817-86.35 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 071. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-071 was assayed for expression of SIV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal goat anti-HA serum was shown to react specifically with S-SPV-071 plaques and not with S-SPV-001 negative control plaques. All S-SPV-071 observed plaques reacted with the goat anti-HA serum indicating that the virus was stably expressing the SIV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

To confirm the expression of the SIV HA and NA gene products, cells were infected with S-SPV-071 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A Polyclonal swine anti-SIV serum or a Polyclonal goat anti-HA serum

was used to detect expression of SIV specific proteins. The cell lysate and supernatant from S-SPV-071 infected cells exhibited bands corresponding to 64 kd and 52 kd, which is the expected size of the SIV HA and NA protein.

S-SPV-071 is useful as a vaccine in swine against SIV infection and is useful for expression of SIV-HA and NA. S-SPV-071 is useful as a vaccine in combination with S-SPV-063 which expresses SIV NP.

S-SPV-074

S-SPV-074 is a swinepox virus that expresses at least four foreign genes. The gene for E. coli β-glucuronidase (uidA) and the genes for swine influenza virus (SIV) HA (H1N1) and NA (H1N1) were inserted into the SPV 617 48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The uidA gene is under the control of the synthetic late/early promoter (LP2EP2), and the SIV HA and NA genes are under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-074 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 817.14.2 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 074. This virus was assayed for β-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue

plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-074 was assayed for expression of SIV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-SIV serum was shown to react specifically with S-SPV-074 plaques and not with S-SPV-001 negative control plaques. All S-SPV-074 observed plaques reacted with the goat anti-HA serum indicating that the virus was stably expressing the SIV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

S-SPV-074 is useful as a vaccine in a swine against SIV infection and is useful for expression of SIV HA and NA. S-SPV-074 is useful as a vaccine in combination with S-SPV-063 which expresses SIV NP. S-SPV-063, -065, -066, -071, and -074, are useful alone or in combination as a vaccine in swine against swine influenza infection and are useful for expression of the SIV NP, HA, and NA proteins.

<u>S-SPV-068:</u>

S-SPV-068 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* ß-galactosidase (lacZ) and the gene for chicken macrophage migration inhibitory factor (cMIF) were inserted into the 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of nucleotides 1679 to 2452, SEQ ID NO: 189).

The lacZ gene is under the control of the swinepox O1L promoter, and the cMIF gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-068 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 802-95.A1 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-068. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

To confirm the expression of the cMIF gene product, cells were infected with S-SPV-068 and samples of infected cell lysates were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal goat anti-human cMIF antibody was used to detect expression of cMIF specific proteins. The cell lysate from S-SPV-068 infected cells exhibited a band corresponding to approximately 15 kd, which is the expected size of the cMIF protein.

S-SPV-068 is useful as a vaccine in chickens to inhibit migration of macrophages and to stimulate an immune response against infection by avian pathogens. S-SPV-068 is useful for expression of cMIF.

HOMOLOGY VECTOR 802-95.A1. The plasmid 802-95.A1 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lac Z) marker gene and an chicken macrophage migration inhibitory factor (cMIF) gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 855 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1113 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß galactosidase (lacZ) marker gene is under the control of a swinepox virus 01L gene promoter and the cMIF gene is under the control of the late/early promoter (LP2EP2). The LP2EP2 cMIF gene cassette was inserted into a BamHI site of homology vector 752-22.1. Homology vector 802-95.A1 was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2519 base pair HindIII to SphI restriction fragment of pSP65 (Promega). Fragment 1 is an approximately 855 base pair sub-fragment of the SPV HindIII restriction fragment M (23) synthesized by polymerase chain reaction using DNA primers 5' GAAGCATGCCCGTTCTTATCAATAGTTTAGTCGAAAATA-3' and 5'-CATAAGATCTGGCATTGTGTTATTATACTAACAAAAATAAG-3' to produce an 855 base pair fragment with SphI and BglII ends. Fragment 2 is a 3002 base pair *Bam*HI to *Pvu*II fragment derived from plasmid pJF751 (49) containing the *E. coli* lacz gene. Fragment 3 is an approximately 363 base pair BglII fragment coding for the cMIF gene (63) derived by reverse transcription and polymerase chain reaction

(PCR) (Sambrook, et al., 1989) of RNA ISOLATED FROM CONCANAVALIN A STIMULATED CHICKEN SPLEEN CELLS. The antisense primer used for reverse transcription and PCR was 5' TCGAAGATCTTCTCATGCAAAGGTGGAACCGTTC -3' (6/95.28; SEQ ID NO: 58). The sense primer used for PCR was 5' TCGAAGATCTCATGCCTATGTTCACCATCCACAC -3' (6/95.27; SEQ ID NO: 59). The DNA fragment contains the open reading frame of 121 amino acids of the chicken macrophage migration inhibitory factor protein. The native methionine codon of cMIF is preceded by amino acid codons for met-asn-ser-asp-lys. Fragment 4 is an approximately 1113 base pair subfragment of the SPV HindIII fragment M synthesized by polymerase chain reaction using DNA primers 5'-CCGTAGTCGACAAAGATCGACTTATTAATATGTATGGGATT-3' (and 5' GCCTGAAGCTTCTAGTACAGTATTTACGACTTTTGAAAT-3' to produce an 1113 base pair fragment with SalI and HindIII ends.

S-SPV-069

S-SPV-069 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli β-galactosidase (lacZ) and the gene for human respiratory syncytial virus (HRSV) fusion (F) protein were inserted into the SPV 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of nucleotides 1669 to 2452, SEQ ID NO: 189). The lacZ gene is under the control of the swinepox P_{O1L} promoter and the HRSV F gene is under the control of the synthetic late/early promoter (LP2EP2). S-SPV-069 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 810-29.A2 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING

β-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 069. This virus was assayed for β-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-069 was assayed for expression of HRSV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Monoclonal antibody 621 (Biodesign, Inc.) against HRSV F was shown to react specifically with S-SPV-069 plaques and not with S-SPV-001 negative control plaques. All S-SPV-069 observed plaques reacted with the monoclonal antibody 621 indicating that the virus was stably expressing the PRV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

S-SPV-078

S-SPV-078 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli β-galactosidase (lacZ) and the gene for human respiratory syncytial virus (HRSV) attachment (G) protein were inserted into the SPV 617 48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late/early promoter (LP2EP2), and the HRSV G gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-078 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 822-52G.7 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock is screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification is the recombinant virus designated S-SPV-078. This virus is assayed for β-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign gene.

S-SPV-069 and S-SPV-078 are useful individually or in combination as a vaccine in swine against human respiratory syncytial virus infection and are useful for expression of HRSV F and G genes.

HOMOLOGY VECTOR 810-29.A2. The plasmid 810-29.A2 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β-galactosidase (lac Z) marker gene and a human respiratory syncytial virus (HRSV) fusion (F) gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 855 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1113 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a swinepox virus 01L gene promoter and the

HRSV F gene is under the control of the late/early promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2519 base pair HindIII to SphI restriction fragment of pSP65 (Promega). Fragment 1 is an approximately 855 base pair sub-fragment of the SPV HindIII restriction fragment M (23) synthesized by polymerase chain reaction using DNA primers 5' GAAGCATGCCCGTTCTTATCAATAGTTTAGTCGAAAATA-3' and 5'-CATAAGATCTGGCATTGTGTTATTATACTAACAAAAATAAG-3' to produce an 855 base pair fragment with SphI and BglII ends. Fragment 2 is a 3002 base pair BamHI to PvuII fragment derived from plasmid pJF751 (49) containing the E. coli lacZ gene. Fragment 3 is an approximately 1728 base pair EcoRI restriction fragment synthesized by reverse transcriptase and polymerase chain reaction (PCR) (15, 42) using RNA from the HRSV Strain A2 (ATCC VR-1302). The primer (5' GCCGAATTCGCTAATCCTCAAAGCAAATGCAAT-3';4/95.23) synthesizes from the 5' end of the HRSV F gene, introduces an EcoRI site at the 5' end of the gene and an ATG start codon. The primer (5'-GGTGAATTCTTTATTTAGTTACTAAATGCAATATTATTT-3'; 4/95.24) synthesizes from the 3' end of the HRSV F gene and was used for reverse transcription and polymerase chain reaction. The PCR product was digested with EcoRI to yield a fragment 1728 base pairs in length corresponding to the HRSV F gene. Fragment 4 is an approximately 1113 base pair subfragment of the SPV HindIII fragment M synthesized by polymerase chain reaction using DNA primers 5'-CCGTAGTCGACAAAGATCGACTTATTAATATGTATGGGATT-3' and 5'

GCCTGAAGCTTCTAGTACAGTATTTACGACTTTTGAAAT-3' to produce an 1113 base pair fragment with SalI and HindIII ends.

HOMOLOGY VECTOR 822-52G.7. The plasmid 822-52G.7 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an *E. coli* β-galactosidase (lacZ) marker gene and the human respiratory syncytial virus (HRSV) attachment (G) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When this plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes will result. Note that the β-galactosidase (lacZ) marker gene is under the control of a synthetic late/early pox promoter (LP2EP2) and the HRSV G gene is under the control of a synthetic late/early pox promoter (LP2EP2).It was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources. The plasmid vector was derived from an approximately 2972 base pair *Hind*III to *BamH*I restriction fragment of pSP65 (Promega). Fragment 1 is an approximately 1484 base pair AccI to BglII restriction sub-fragment of the SPV *Hind*III fragment M (23). Fragment 2 is an approximately 3006 base pair *Bam*HI to *Pvu*II restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 899 base pair EcoRI restriction fragment synthesized by reverse transcriptase and polymerase chain reaction (PCR) (15, 42) using RNA from the HRSV Strain A2 (ATCC VR-1302). The primer (5' GCC<u>GAATTC</u>CAAAAACAAGGACCAACGCAC-3';4/95.25) synthesizes from the 5' end of the HRSV F gene, introduces an EcoRI site at the 5' end of the

gene and an ATG start codon. The primer (5'-GCCGAATTCACTACTGGCGTGGTGTGTTG-3'; 4/95.26) synthesizes from the 3' end of the HRSV G gene and was used for reverse transcription and polymerase chain reaction. The PCR product was digested with EcoRI to yield a fragment 899 base pairs in length corresponding to the HRSV G gene. Fragment 4 is an approximately 2149 base pair HindIII to *Acc*I restriction sub-fragment of the SPV *Hind*III restriction fragment M (23).

HOMOLOGY VECTOR 807-41.3. The plasmid 807-41.3 was used to insert foreign DNA into SPV. It incorporates an E. coli B-galactosidase (lacZ) marker gene and the swine influenza virus (SIV) nucleoprotein (NP) gene flanked by SPV DNA. When this plasmid was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the B galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1) and the SIV NP gene is under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair Hindiii to Bam HI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base Bglii to AccI restriction sub-fragment of the SPV HindIII fragment M(23). Fragment 2 is an approximately 1501 base pair EcoRI to EcoRI fragment of the SIV NP gene synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using RNA from the SIV H1N1 strain (NVSL). The primer (5'CATGAATTCTCAAGGCACCAAACGATCATATGAAC-3'; 6/95.13) synthesizes from the 5' end of the SIV NP gene and introduces an EcoRI site at the 5'-

ATTTGAATTCAATTGTCATACTCCTCTCGCATTGTCT-3';6/95.14) synthesizes from the 3' end of the SIV NP gene, introduces an EcoRI site st the 3' end of the gene, and was used for reverse transcription and polymerase chain reaction. The PCR product was digested with EcoRI to yield a fragment 1501 base pairs in length corresponding to the SIV NP gene. Fragment 3 is approximately 3010 base pair BamHI to PuvII restriction fragment of plasmid pJF751 (11). Fragment 4 is approximately 2149 base pair AccI to HindIII restriction sub-fragment of the SPV Hind III restriction fragment M (23)

HOMOLOGY VECTOR 807-84.8. The plasmid 807-84.8 was used to insert foreign DNA into SPV. It incorporates an E. coli B-galactosidase (lacZ) marker gene and the swine influenza virus (SIV) hemmagglutinin (HA) gene flanked by SPV DNA. When this plasmid was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the B-galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1) and the SIV HA gene is under the control of a synthetic late/early promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restricting fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI resriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment fo the SPV HindIII fragment M(23). Fragment 2 is an approximately 1721 base pair BamHI to BamHI gragment of the SIV HA gene synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using RNA from the SIV H1N1 strain (NVSL). The primer

(5'CCGAGGATCCGGCAATACTATTAGTCTTGCTATGTACAT-3'; 6/95.5) synthesizes from the 5' end of the SIV HA gene and introduces an BamHI site at the 5; end of the gene.The primer (5'- CTCTGGATCCTAATTTAAATACATATTCTGCACTGTS-3'; 6/95.6) synthesizes from the 3' end of the SIV HA gene, introduces a Bam HI site at the 3' end of the gene, and was used for the reverse transcription and polymerase chain reaction. The PCR product was idgested with EcoRI to yield a fragment 1721 base pairs in length corresponding to the SIV HA gene. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair AccI to fragmeny M (23).

HOMOLOGY VECTOR 807-84.35. The plasmid 807-84.35 was used to insert foreign DNA into SPV. It incorporates an E. coli B-galactosidase (lacZ) marker gene and the swine influenza virus (SIV) neuraminidase (NA) gene flanked by SPV DNA. When this PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the B-galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1) and the SIV NA gene isunder the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30) by joining restricting fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII fragment M (23). Fragment 2 is an approximately 1414 base pair EcoRI to BglII fragment of the SIV NA gene synthesized by reverse transcription (RT) and polymerase chain reaction)(PCR) (15,42) using RNA from the SIV H1N1 strain (NVSL). The primer (5'

AATGAATTCAAATCAAAAAATAATAACCATTGGGTCAAT-3'; 6.95.12) synthesizes from the 3' end of the SIV NA gene, introduces an EcoRI site at the 5' end of the gene. The primer (5'-GGAAGATCTACTTGTCAATGGTGAATGGCAGATCAG-3';6/95.13) synthesizes from the 3' end of the SIV NA gene, introduces an BglII site at the 3' end of the gene, and was used for reverse transcription and polymerase chain reaction. The PCR product was digested with EcoRI to yield a fragment 1414 base pairs in length corresponding to the SIV NA gene. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair AccI to HindIII restriction sub-fragment of the SPV HindIII restriction fragment M (23).

HOMOLOGY VECTOR 807-86.35. The plasmid 807-86.35 was used to insert foreign DNA into SPV. It incorporates an E. coli B-galactosidase (lacZ) marker gene and the swine influenza virus (SIV) HEMAGGLUTININ (HA) and neuraminidae (NA) gene flanked by SPV DNA. When this plasmid was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the B-galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1) and the SIV NA and HA genes are each under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII fragment M (23).

Fragment 2 is an approximately 1721 base pair BamHI to BamHI fragment fo the SIV HA gene synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using RNA from the SIV H1N1 strain (NVSL). The primer (5'-CCGAGGATCCGGCAATACTATTAGTCTTGCTATGTACAT-3';6/95.5 synthesizes from the 5' end of the SIV HA gene and introduces an Bam HI site at the 5' end of the gene. The primer (5'-CTCTGGGATCCTAATTTTAAATACATATTCTGCACTGTA-3'; 6/95.6) synthesizes from the 3' end of the SIV HA gene, introduces an BamHI site at the 3' end of the gene, introduces an BamHI site at the 3' end of the gene, and was used for reverse transcription and polymerase chain reaction. The PCR product was digested with EcoRI to yield a fragment 1721 base pairs in length corresponding to the SIV HA gene. Fragment 3 is an approximately 1414 base pair EcoRI to BglII fragment of the SIV NA gene synthesized by reverse transcription (RT)_and polymerase chain reaction (PCR) (15,42) using RNA from the SIV H1N1 strain (NVSL). The primer (5' AATGAATTCAAATCAAAAAATAATAACCATTGGGTCAAT-3';6/95.12) synthesizes from the 5' end of the SIV NA gene and introduces an EcoRI site at the 5' end of the gene. The primer (5' - GGAAGATCTACTTGTCAATGGTGAATGGCAGATCAG-3'; 6/95.13) synthesizes from the 3' end of the SIV NA gene, introduces an BglII site at the 3' end of the gene, and was used for reverse transcription and polymerase chain reaction. The PCR product was digested with EcoRI to yield a fragment 1414 base pairs in length corresponding to the SIV NA gene. Fragment 4 is an approximately 2149 base pair AccI to HindIII restriction sub-fragment of the SPV HindIII restriction fragment M (23).

HOMOLOGY VECTOR 817-14.2. The plasmid 817-14.2 was used to insert foreign DNA into SPV. It incorporates an E.

coli B-galactosidase (lacZ) marker gene and the swine influenza virus (SIV) HEMAGGLUTININ (HA) and neuraminidae (NA) gene flanked by SPV DNA. When this plasmid was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the B-galactosidase (uida) marker gene is under the control of a synthetic late/early pox promoter (LP2EP2) and the SIV NA and HA genes are each under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction subfragment to the SPV HindIII fragment M (23). Fragment 2 is an approximately 1721 base pair BamHI to BamHI fragment of the SIV HA gene synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using RNA from the SIV H1N1 strain (NVSL). The primer (5' CCGAGGATCCGGCAATACTATTAGTCTTGCTATGTACAT-3';6/95.5) synthesizes from the 5' end of the SIV HA gene and introduces an BamHI site at the 5' end of the gene. The primer (5'-CTCTGGGATCCTAATTTTAAATACATATTCTGCACTGTA-3'; 6/95.6) synthesizes from the 3' end of the SIV HA gene, introduces an BamHI site at the 3' end of the gene, and was used for reverse transcription and polymerase chain reaction. The PCR product was digested with EcoRI to yield a fragment 1721 base pairs in length corresponding to the SIV HA gene. Fragment 3 is an approximately 1414 base pair EcoRI to BglII fragment of the SIV HA gene synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using

RNA for the SIV H1N1 strain (NVSL). The primer (5' AATGAATTCAAATCAAAAAATAATAACATTGGGTCAAT-3';6/95.12) synthesizes from the 5' end of the SIV NA gene, introduces an EcoRI site at the 5' end of the gene. The primer (5'-GGAAGATCTACTTGTCAATGGTGAATGGCAGATCAG-3';6/95.13) synthesizes from the 3' end of the SIV NA gene, introduces an BglII site at the 3' end of the gene, and was used for reverse transcription and polymerase chain reaction. The PCR product was digested with EcoRI to yield a fragment 1414 base pairs in length corresponding to the SIV NA gene. Fragment 4 is an approximately 1823 base pair NotI restriction fragment of plasmid pRAJ260 (Clonetech). Fragment 5 is an approximately 2149 base pair AccI to HindIII restriction sub-fragment of the SPV HindIII restriction fragment M (23).

PRRS HOMOLOGY VECTORS CONTAINING SINGLE OR MULTIPLE PRRS GENES (ORF2, ORF3, ORF4, ORF5, ORF6 or ORF7: The PRRS homology vector is constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli B-galactosidase (lacZ) marker gene and a porcine reproductive and respiratory syndrome virus (PRRS) ORF2, ORF3, ORF4, ORF5, ORF6 or ORF7 gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 855 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1113 base pair frament of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the B-galactosidase (lacZ) marker gene is under the control of a swinepox virus 01L gene promoter and the PRRS gene is under the control of the late/early promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA sequences. The plasmid vector was derived from an

approximately 2519 base pair HINDIII to SphI restriction fragment of pSP65 (Promega). Fragment 1 is an approximately 855 base pair sub-fragment of the SPV polymerase chain reaction using DNA primers 5' GAAGCATGCCCGTTCTTATCAATAGTTTAGTCGAAAATA-3' and 5'-CATAAGATCTGGCATTGTGTTATTATACTAACAAAAATAAG-3' to produce an 855 base pair fragment with SphI and BglII ends. Fragment 2 is a 3002 base pair BamHI to PvuII fragment derived from plasmid pJF751 (49) containing the E. coli lacZ gene. Fragment 3 is an EcoRI to BamHI restriction fragment synthesized by reverse transcription and polymerase chain reaction (PCR) using genomic RNA from a U.S. Isolate of PRRS obtained from the NVSL (Reference strain). Each homology vector contains one or multiple of the PRRS virus ORF2 through 7. To synthesize PRRS ORF2, the primer (5' AATGAATTCGAAATGGGTCCATGCAAAGCCTTTTTG-3';1/96.15) synthesizes from the 5' end of the PRRS ORF2 gene, introduces an EcoRI site at the 5' end of the gene. The primer (5'- CAAGGATCCCACACCGTGTAATTCACTGTGAGTTCG-3'; 1/96.16) is used for reverse transcription and PCR and synthesizes from the 3' end of the PRRS ORF2 gene. The PCR product was digested with EcoRI and BamHI to yield a fragment 771 base pairs in length corresponding to the PRRS ORF2 gene. To synthesize PRRS ORF3, the primer (5' TTCGAATTCGGCTAATAGCTGTACATTCCTCCATATTT-3'; 1/96.7) synthesizes from the 5' end of the PRRS ORF3 gene, introduces an EcoRI site at the 5' end fo the gene. The primer (5'- GGGGATCCTATCGCCGTACGGCACTGAGGG-3'; 1/96.8) is used for reverse transcription and PCR and synthesizes from the 3' end of the PRRS ORF3 gene. To synthesize PRRS ORF4, the primer (5' CCGAATTCGGCTGCGTCCCTTCTTTTCCTCATGG-3'; 1/96.11) synthesizes from the 5' end of the PRRS ORF4 gene, introduces an EcoRI site at the 5'-CTGGATCCTTCAAATTGCCAACAGAATGGCAAAAAGAC-3'; 1/96.12) is used for reverse transcription and PCR and synthesizes

from the 3' end of the PRRS ORF4 gene. The PCR product was digested with EcoRI adn BamHI to yield a fragment 537 base pairs in length corresponding to the PRRS ORF4 gene. To synthesize PRRS ORF5, the primer (5' TTGAATTCGTTGGAGAAATGCTTGACCGCGGGC-3'; 1/96.13) synthesizes from the 5' end of the PRRS ORF5 gene, introduces an EcoRI site at the 5' end of the gene. The primer (5'- GAAGGATCCTAAGGACGACCCCATTGTTCCGCTG-3';1/96.14)is used for reverse transcription and PCR and synthesizes from the 3' end of the PRRS ORF5 gene. The PCR product was digested with EcoRI and BamHI to yield a fragment 603 base pairs in length corresponding to the PRRS ORF5 gene. To synthesize PRRS ORF6, the primer (5' CGGGAATTCGGGGTCGTCCTTAGATGACTTCTGCC-3'; 1/96.17) synthesizes from the 5' end of the PRRS ORF6 gene, introduces an EcoRI site at the 5' end of hte gene. The primer (5' -GCGGATCCTTGTTATGTGGCATATTTGACAAGGTTTAC-3'; 1/96.18) is used for reverse transcription and PCR and synthesize from the 3' end of the PRRS ORF6 gene. The PCR product was digested with EcoRI and BamHI to yield a fragment 525 base pairs in length corresponding to the PRRS ORF6 gene. To synthesize PRRS ORF7, the primer (5' GTCGAATTCGCCAAATAACAACGGCAAGCAGCAGAAG-3'; 1/96.19) synthesizes from the 3' end of the PRRS ORF7 gene. Fragment 4 is an approximately 1113 base pair subfragment of the SPV HindIII fragment M synthesized by polymerase chain reaction using DNA primers 5'-CCGTAGTCGACAAAGATCGACTTATTAATATGTATGGGATT-3' and 5' GCCTGAAGCTTCTAGTACAGTATTTACGACTTTTGAAT-3' to produce and 1113 base pair fragment with SalI and HindIII ends.

Recombinant swinepox virus expressing pseudorabies genes

S-SPV-076 is a swinepox virus that expresses at least three foreign genes. The gene for E. coli B-galactosidase (lacZ) and the genes for pseudorabies virus (PRV) gD and gI were inserted into the SPV 617 48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the PRV gD and gI genes are under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-077 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli B-galactosidase (lacZ) and the gene for pseudorabies virus (PRV) gI were inserted into the SPV 617 48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the PRV gI gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-079 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli B-galactosidase (lacZ) and the gene for pseudorabies virus (PRV) gI were inserted into the SPV 617 48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the PRV gB gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-076, S-SPV-077, S-SPV-079 have been tested by BLACK PLAQUE ASSAY and WESTERN BLOT for expression of the PRV glycoproteins.

S-SPV-076, S-SPV-077, and S-SPV-079 were derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing a homology vector and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock were screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING B-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-076, S-SPV-077, and S-SPV-079. The viruses were assayed for B-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-076, S-SPV-077, and S-SPV-079 are useful as a vaccine in swine against PRV infection and is useful for expression of PRV gD, gI or gB. S-SPV-071 is useful as a vaccine in combination with a recombinant swinepox virus which expresses PRV gC, such as S-SPV-011, S-SPV-012 or S-SPV-013.

143B	carcinoma* osteosarcoma*
A431	epidermoid carcinoma*
A549	lung carcinoma*
Capan-1	liver carcinoma*
CF500	foreskin fibroblasts
Chang Liver	liver
Detroit	Downs'foreskin fibroblasts
HEL-199	embryonic lung
HeLa	cervical carcinoma*
Hep-2	epidermal larynx carcinoma
HISM	intestinal smooth muscle

HNK	neonatal kidney
MRC-5	embryonic lung
NCI-H292	pulmonary mucoepidermoid
OVCAR-3	ovarian carcinoma*
RD	rhabdosarcoma*
THP	monocyte (leukemia)*
WIL2-NS	B lymphocyte line, non-secreting
WISH	amnion
PBL	peripheral blood lymphocytes

Example 38

Recombinant swinepox virus expressing PRRS genes ORF2, ORF3, ORF4, ORF5 and ORF6

S-SPV-080 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli B-galactosidase (lacZ) and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF2 were inserted into the SPV 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of nucleotides 1669 to 2452, (SEQ ID NO: 189). The lacZ gene is under the control of the swinepox P_{O1L} promoter and the PRRS ORF2 gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-081 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli B-galactosidase (lacZ) and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF3 were inserted into the SPV 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of nucleotides 1669 to 2452, (SEQ ID NO: 189). The lacZ gene is under the control of the swinepox P_{O1L} promoter and the PRRS ORF3 gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-082 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli B-galactosidase (lacZ) and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF4 were inserted into the SPV 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of nucleotides 1669 to 2452, (SEQ ID NO: 189). The lacZ gene is under the control of the swinepox P_{O1L} promoter and the PRRS ORF4 gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-083 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli B-galactosidase (lacZ) and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF5 were inserted into the SPV 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of nucleotides 1669 to 2452, (SEQ ID NO: 189). The lacZ gene is under the control of the swinepox P_{O1L} promoter and the PRRS ORF5 gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-084 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli B-galactosidase (lacZ) and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF6 were inserted

into the SPV 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of nucleotides 1669 to 2452, (SEQ ID NO: 189). The lacZ gene is under the control of the swinepox P_{O1L} promoter and the PRRS ORF6 gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-085 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli B-galactosidase (lacZ) and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF7 were inserted into the SPV 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of nucleotides 1669 to 2452, (SEQ ID NO: 189). The lacZ gene is under the control of the swinepox P_{O1L} promoter and the PRRS ORF7 gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-080, S-SPV-081, S-SPV-082, S-SPV-083, S-SPV-084, S-SPV-085 were derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector Material and Methods (PRRS HOMOLOGY VECTORS) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING B-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-080, S-SPV-081, S-SPV-082, S-SPV-083, S-SPV-084, S-SPV-085. This virus was assayed for B-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-080, S-SPV-081, S-SPV-082, S-SPV-083, S-SPV-084, S-SPV-085 are useful individually or in combination as vaccines in swine against PRRS infection and are useful for expression of PRRS ORF2, ORF3, ORF4, ORF5, ORF6 and ORF7.

Example 39

The following experiment was performed to determine the ability of swinepox virus to infect human cells in culture and express a foreign DNA as lacZ.

S-SPV-003 was absorbed to the human cell lines listed in the Table below at an MOI=0.1 for 2 to 3 hours. Cells were rinsed three times with PBS, growth media was added, and cells were incubated at 37°C for four days. Cells were harvested and a lysate prepared in 200 microliters of PBS by freeze/thaw three times. Cell debris was pelleted, and 10 microliters of supernatant was assayed for -galactosidase activity by ONPG assay at 37°C for 1 1/2 hours. The table shows the results of infection of various human cell lines with S-SPV-003 and the relative levels of cytopathetic effect and expression of lacZ.

The results show that various human cell lines vary in the ability to take up S-SPV-003 and express lacZ. CPE was minimal in all cases and did not result in viral replication. One exception A549 cells which did show some rounding of cells and lifting off the plate in one instance, and another instance of ten-fold increase in titer during passage suggesting limited viral replication. Several cell lines how significant lacZ activity with no cytopathetic effect.

Different pox promoters express lacZ from recombinant swinepox virus in a number of human cell lines. Six

different swinepox viruses were constructed which expressed lacZ from EP1, LP1, LP2, EP1LP2, LP2EP2, or the SPV PO1L promoter. Viruses were each used to infect A549, Chang liver, or 143B cells at o.1 moi, and cells were rinsed between 2 and 3 hours later and then incubated for 4 days at 37°C. Each cell line maintained a different hierarchy of promoter activity, which was reproducible in following experiments.

For example, the EP1, LP2EP2, and PO1L promoters gave the most expression in 143B cells, while the LP2 was strongest in Chang liver cells, and the EP1LP2 in A549. In the Chang liver and A549 cells, expression from the PO1L promoter was poorest, whereas in 143B, espression from LP2 was poorest. Therefore, different human cell lines utilize pox promoters in dissimilar ways. This may reflect how far the swinepox virus can proceed along the replication pathway in different cell lines.

These early and late promoters exhibited lower or higher lacZ activity depending on the human cell type infected by the recombinant swinepox virus. By choosing different promoters for different target tissues, one is able to regulate the amounts of foreign gene product delivered by the swinepox virus to target tissues.

Recombinant swinepox virus is useful as a vaccine for human infectious disease and to deliver therapeutic agents to humans. Recombinant swinepox virus is useful as a vaccine against viral or bacterial infection in humans, and as a therapeutic for cancer or genetic disease to deliver antibodies, tumor antigens, cell surface ligands and receptors, immune modulating molecules such as cytokines

Example 40

S-SPV-003 Expression of lacZ in human cell lines
Measurement of cytopathic effect and lacZ expression

Cell Type	Cytopathetic Effect*	LacZ Expression**
A431 epidermoid carcinoma*	———	———
A549 lung carcinoma*	++	+++
Capan-1 liver carcinoma*	———	———
CF500 foreskin fibroblasts	+	+
Chang Liver	+	+++
Detroit Down's foreskin fibroblasts	+/-	———
HEL-199 embryonic lung	+/-	+++
HEp-2 epidermal larynx carcinoma*	———	———
HISM intestinal smooth muscle	+	+
HNK neonatal kidney	———	++
MRC-5 embryonic lung	+/-	+
NCI-H292 pulmonary mucoepidermoid carcinoma*	———	+++

OVCAR-3 ovarian carcinoma*	———	+++
RD rhabdosarcoma*	———	+
THP monocyte (leukemia)*	———	+
WIL2-NS B lymphocyte line, non-secreting	———	———
WISH amnion	+/-	++
HeLa	———	+++
PBL peripheral blood lymphocytes	———	———

* When human cells are infected with SPV, a cytopathic effect is sometimes seen. In most cell lines, this cytopathic effec is evidenced by a chang in the appearance of the cells, with cells becoming thinner and more ragged along the edges; cells look stressed. This phenomenon was assessed as follows:

\- indicates no difference between infected & uninfected cells;

+/- indicates that the monolayer is visibly different from uninfected, though most cells appear normal;

\+ indicates that the monolayer is obviously affected, with most cells looking stressed. It should be noted that in certain cell lines (HeLa, CF500, 143B), in which titers were obtained after serial passage, there was no evidence for replication of SPV, with one exception.

A549 was given a ++ for cytopathic effect in one instance, when cells appeared to round up and come off the plate during infection, though this observation was not repeated. A549 also showed evidence in another case of a ten-fold increase in titer during passage, suggesting that it might support limited viral replication.

**	B-galactosidase activity in A_{260} units per cell lysate from 1/20 of a 35 mm dish:
-	No activity
+	0.2-0.9 A_{260} unit
++	0.9-1,6 A_{260} unit
+++	greater than 1.6 A_{260} units.

Example 41: BOVINE CONSTRUCTS AND VACCINES

S-SPV-112

S-SPV-112 is a swinepox virus that expresses three foreign genes. The gene for E. coli ß-galactosidase (lacZ) and the gene for bovine respiratory syncytial virus (BRSV) attachment (G) were inserted into the unique NotI restriction site (NotI linkers inserted into a unique NdeI site in the SPV O1L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted). The gene for BRSV fusion (F) was inserted into the unique PstI restriction site (PstI linkers inserted into a

unique AccI site in the SPV 01L open reading frame). The lacZ gene is under the control of the synthetic late promoter (LP1), the BRSV G and F genes are each under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-112 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 848-02 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 112. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-112 is useful as a vaccine in bovine against disease caused by bovine respiratory syncytial virus. The BRSV antigens are key to raising a protective immune response in the animal. The recombinant viruses are useful alone or in combination as an effective vaccine. The swinepox virus is useful for cloning other subtypes of BRSV to protect against rapidly evolving variants in this disease. S-SPV-112 is also useful as an expression vector for expressing BRSV antigens. Such BRSV antigens are useful to identify antibodies directed against the wild-type BRSV. The virus is also useful as a source of antigens for the production of monospecific polyclonal or monoclonal antibodies. Such

antibodies are useful in the development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

HOMOLOGY VECTOR 848-02. The plasmid 848-02 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene, the bovine respiratory syncytial virus (BRSV) attachment (G) and fusion (F) genes flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the BRSV F and G genes are under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 1722 base pair Bam HI fragment generated by PCR which contains the coding sequence of the BRSV F gene. Fragment 3 is an approximately 48 base pair AccI to

NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 771 base pair Bam HI fragment generated by PCR which contains the coding sequence for the BRSV G gene. The BRSV F and G genes were synthesized by PCR as described in the CLONING OF BOVINE RESPIRATORY SYNCYTIAL VIRUS FUSION, NUCLEOCAPSID AND GLYCOPROTEIN GENES. Fragment 5 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 6 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique PstI sites using PstI linkers. The NdeI sites in fragments 3 and 6 were converted to unique Not I sites using NotI linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted which would span SPV fragments 3 and 6.

<u>Recombinant swinepox virus expressing BRSV F and G fusion protein</u>

<u>S-SPV-130:</u>

S-SPV-130 is a swinepox virus that expresses three foreign genes. The gene E. coli ß-galactosidase (lacZ) is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The genes for bovine respiratory syncytial virus (BRSV) attachment (G) and BRSV fusion (F) are inserted into the unique NotI restriction site (NotI linkers inserted into a unique NdeI site in the SPV O1L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to

2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted). The lacZ gene is under the control of the synthetic late promoter (LP1), the BRSV F/G fusion gene is under the control of the synthetic late/early promoter (LP2EP2). The BRSV F/G fusion gene comprises approximately 1560 nucleotides of the F gene (520 amino acids including the amino terminus) fused in frame to approximately 580 nucleotides of the G gene (193 amino acids including the carboxy terminus).

S-SPV-130 is derived from S-SPV-001 (Kasza Strain). This is accomplished utilizing the HOMOLOGY VECTOR 807-75.41 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock is screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The recombinant virus is isolated by red plaque purification. This virus is assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-130 is useful as a vaccine in bovine against disease caused by bovine respiratory syncytial virus. The BRSV F/G fusion protein is particularly effective and key to raising a protective immune response in the animal. The BRSV F/G fusion protein contains the intact amino terminus of the F protein and the intact carboxy terminus of the G protein which includes the known immunogenic region of each protein. The BRSV F/G fusion protein provides an improved immune response compared

to expressing the BRSV F and G proteins separately or expressing isolated epitopes of the BRSV F and G proteins. The swinepox virus is useful for cloning other subtypes of BRSV to protect against rapidly evolving variants in this disease. Recombinant swinepox virus is also useful as an expression vector for expressing BRSV antigens. Such BRSV antigens are useful to identify antibodies directed against the wild-type BRSV. The virus is also useful as a source of antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

HOMOLOGY VECTOR 807-75.41. The homology vector 807-75.41 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene, a fusion protein of the bovine respiratory syncytial virus (BRSV) attachment (G) and fusion (F) genes flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the BRSV F/G fusion gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30),

by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector is derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 1560 base pair Bam HI fragment generated by PCR which contains the coding sequence of the BRSV F gene. The F gene coding region from the BRSV strain 375 (VR-1339) was cloned using the following primers: 5'-GCGGATCCGGCGCGCCGGATTTTCCTACATCTACACT-3' (5/96.26; **SEQ ID NO 12**) for cDNA priming and combined with 5'-CTAAAATTGAATTGTAAT-3' (1/95.19; **SEQ ID NO 13**:) for PCR. The DNA encodes 520 amino acids at the amino terminus of the BRSV F protein. Fragment 5 is an approximately 580 base pair AscI fragment generated by PCR which contains the coding sequence for the BRSV G gene. The G gene coding region from the BRSV strain 375 (VR-1339) was cloned using the following primers: 5' TTGGCGCGCCCTAGATCTGTGTAGTTGATTGATTTG-3' (5/96.28; **SEQ ID NO 14**:) for cDNA priming and combined with 5' TACGGCGCGCCGGGAAATGCTAAAGCCAAGCCCACA-3' (5/96.27; **SEQ ID NO 15**:) for PCR. The DNA product encodes 193 amino acids (including a translation stop codon) of the carboxy terminus of the BRSV G protein. The BRSV F and G coding sequences are fused in the correct translational reading frame. Fragment 6 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI

sites in fragments 1 and 3 were converted to unique PstI sites using PstI linkers. The NdeI sites in fragments 3 and 6 were converted to unique Not I sites using NotI linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted which would span SPV fragments 3 and 6.

S-SPV-099

S-SPV-099 is a swinepox virus that expresses two foreign genes. The gene E. coli ß-galactosidase (lacZ) is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The gene for bovine viral diarrhea virus type 2 (BVDV-2) (strain 890) glycoprotein 53 (gp53) was inserted into the unique NotI restriction site (NotI linkers inserted into a unique NdeI site in the SPV O1L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted). The lacZ gene is under the control of the synthetic late promoter (LP1), the BVDV-2 gp53 gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-099 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 815-73.16A (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant

virus designated S-SPV 099. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-099 is useful as a vaccine in bovine against disease caused by bovine viral diarrhea virus. The BVDV-2 gp53 antigen is key to raising a protective immune response in the animal. The recombinant virus is useful alone or in combination as an effective vaccine. S-SPV-099 is also useful as an expression vector for expressing BVDV antigens. Such BVDV antigens are useful to identify antibodies directed against the wild-type BVDV. The virus is also useful as a source of antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

HOMOLOGY VECTOR 815-73.16A. The homology vector 815-73.16A was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and bovine viral diarrhea virus type 2 (BVDV-2) glycoprotein 53 (gp53) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION

PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), theBVDV gp53 gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 1113 base pair EcoRI/BamHI fragment generated by PCR which contains the coding sequence of the BVDV gp53 gene. BVDV gp53 gene coding region was cloned by reverse transcription and PCR using RNA from BVDV type 2 (Strain 890) as an RNA template for reverse transcription and the following PCR using primers: 5'-TTCGGATCCTGCTCAGACAGTATTGTGTATGTTATCAAGAGC-3' (2/96.32; **SEQ ID NO 16:**) at the 3' end of the BVDV gp53 gene for reverse transcription and PCR combined with 5'-CCATGAATTCCTTCCCTGAATGCAAGGAGGGCTTC-3' (2/96.15; **SEQ ID NO 17:**) at the 5' end of the BVDV gp53 gene for PCR. The DNA encodes approximately 373 amino acids of the BVDV gp53 protein. Fragment 5 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and

3 were converted to unique PstI sites using PstI linkers. The NdeI sites in fragments 3 and 5 were converted to unique Not I sites using NotI linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted which would span SPV fragments 3 and 5.

S-SPV-109

S-SPV-109 is a swinepox virus that expresses two foreign genes. The gene E. coli ß-galactosidase (lacZ) is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The gene for infectious bovine rhinotracheitis virus (IBRV) glycoprotein D (gD) was inserted into the unique HindIII restriction site (HindIII linkers inserted into a unique NdeI site in the SPV O1L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted). The lacZ gene is under the control of the synthetic late promoter (LP1), the IBRV gD gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-109 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 835-57.5 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 109. This virus was assayed for

ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-109 is useful as a vaccine in bovine against disease caused for infectious bovine rhinotracheitis virus. The IBRV gD antigen is key to raising a protective immune response in the animal. The recombinant viruses are useful alone or in combination as an effective vaccine. S-SPV-109 is also useful as an expression vector for expressing IBRV antigens. Such IBRV antigens are useful to identify antibodies directed against the wild-type IBRV. The virus is also useful as a source of antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

HOMOLOGY VECTOR 835-57.5. The homology vector 835-57.5 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and the infectious bovine rhinotracheitis virus (IBRV) glycoprotein D (gD) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION

PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the IBRV gD gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 1320 base pair EcoRI/BamHI fragment generated by PCR which contains the coding sequence of the IBRV gD gene. The IBRV gD gene coding region was cloned by PCR using the HindIII K fragment of the IBRV Cooper strain (pSY 524) as DNA template and the following PCR primers: 5'-CG<u>GGATCC</u>TCACCCGGGCAGCGCGCTGTA-3' (4/96.12; **SEQ ID NO 18:**) at the 3' end of the IBRV gD gene and combined with 5'- CG<u>GAATTC</u>ACAAGGGCCGACATTGGCC-3' (4/96.11; **SEQ ID NO 19:**) at the 5' end of the IBRV gD gene. The DNA encodes approximately 440 amino acids of the IBRV gD protein. Fragment 5 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique PstI sites using PstI linkers. The NdeI sites in fragments 3 and 5 were converted to unique Not I

sites using NotI linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted which would span SPV fragments 3 and 5.

S-SPV-110

S-SPV-110 is a swinepox virus that expresses two foreign genes. The gene E. coli ß-galactosidase (lacZ) is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The gene for infectious bovine rhinotracheitis virus (IBRV) glycoprotein I (gI) was inserted into the unique HindIII restriction site (HindIII linkers inserted into a unique NdeI site in the SPV 01L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted). The lacZ gene is under the control of the synthetic late promoter (LP1), the IBRV gI gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-110 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 835-58.5 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 110. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods.

After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-110 is useful as a vaccine in bovine against disease caused for infectious bovine rhinotracheitis virus. The IBRV gI antigen is key to raising a protective immune response in the animal. The recombinant viruses are useful alone or in combination as an effective vaccine. S-SPV-110 is also useful as an expression vector for expressing IBRV antigens. Such IBRV antigens are useful to identify antibodies directed against the wild-type IBRV. The virus is also useful as a source of antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

HOMOLOGY VECTOR 835-58.5. The homology vector 835-58.5 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and the infectious bovine rhinotracheitis virus (IBRV) glycoprotein I (gI) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß galactosidase (lacZ) marker

gene is under the control of a synthetic late pox promoter (LP1), the IBRV gI gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 1140 base pair EcoRI/BamHI fragment generated by PCR which contains the coding sequence of the IBRV gI gene. The IBRV gI gene coding region was cloned by PCR using the HindIII K fragment of the IBRV Cooper strain (pSY 524) as DNA template and the following PCR primers: 5'-ATCGGGATCCCGTTATTCTTCGCTGATGGTGG-3' (4/96.18; **SEQ ID NO 20**) at the 3' end of the IBRV gI gene and combined with 5'-ATCGGAATTCGCGGTGCCTGTTGCTCTGGATG-3' (4/96.17; **SEQ ID NO 21**) at the 5' end of the IBRV gI gene. The DNA encodes approximately 380 amino acids of the IBRV gI protein. Fragment 5 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique PstI sites using PstI linkers. The NdeI sites in fragments 3 and 5 were converted to unique Not I sites using NotI linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to

2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted which would span SPV fragments 3 and 5.

S-SPV-111

S-SPV-111 is a swinepox virus that expresses two foreign genes. The gene E. coli ß-galactosidase (lacZ) is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The gene for infectious bovine rhinotracheitis virus (IBRV) glycoprotein B (gB) was inserted into the unique NotI restriction site (NotI linkers inserted into a unique NdeI site in the SPV O1L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted). The lacZ gene is under the control of the synthetic late promoter (LP1), the IBRV gB gene is under the control of the synthetic late/early promoter (LP2EP2). The direction of transcription of the IBRV gB gene is opposite the direction of transcription of the lacZ gene and the SPV O1L gene.

S-SPV-111 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 847-15.1C (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 111. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods.

After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-111 is useful as a vaccine in bovine against disease caused for infectious bovine rhinotracheitis virus. The IBRV gB antigen is key to raising a protective immune response in the animal. The recombinant viruses are useful alone or in combination as an effective vaccine. S-SPV-111 is also useful as an expression vector for expressing IBRV antigens. Such IBRV antigens are useful to identify antibodies directed against the wild-type IBRV. The virus is also useful as a source of antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

HOMOLOGY VECTOR 847-15.1C. The homology vector 847-15.1C was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and the infectious bovine rhinotracheitis virus (IBRV) glycoprotein B (gB) gene flanked by SPV DNA. The direction of transcription of the IBRV gB gene is opposite the direction of transcription of the lacZ gene and the SPV O1L gene. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR

GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the IBRV gB gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 2800 base pair EcoRI/BamHI fragment generated by PCR which contains the coding sequence of the IBRV gB gene. The IBRV gB gene coding region was cloned by PCR using the HindIII A fragment of the IBRV Cooper strain (pSY 830-71) as DNA template and the following PCR primers: 5'-CTTCGGATCCTCATGCCCCCCGACGTCGGCCATC-3' (4/96.15; **SEQ ID NO 22**) at the 3' end of the IBRV gB gene and combined with 5'- TCATGAATTCGGCCGCTCGCGGCGGTGCTGAACGC-3' (4/96.10; **SEQ ID NO 23**) at the 5' end of the IBRV gB gene. The DNA encodes approximately 932 amino acids of the IBRV gB protein. Fragment 5 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique PstI sites using PstI linkers. The NdeI sites in fragments 3 and 5 were converted to unique Not I sites using NotI linkers. An

approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted which would span SPV fragments 3 and 5.

S-SPV-113

S-SPV-113 is a swinepox virus that expresses two foreign genes. The gene E. coli ß-galactosidase (lacZ) is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The gene for infectious bovine rhinotracheitis virus (IBRV) glycoprotein C (gC) was inserted into the unique NotI restriction site (NotI linkers inserted into a unique NdeI site in the SPV O1L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted). The lacZ gene is under the control of the synthetic late promoter (LP1), the IBRV gC gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-113 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 848-08 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 113. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue

plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-113 is useful as a vaccine in bovine against disease caused by infectious bovine rhinotracheitis virus. The IBRV gC antigen is key to raising a protective immune response in the animal. The recombinant viruses are useful alone or in combination as an effective vaccine. S-SPV-113 is also useful as an expression vector for expressing IBRV antigens. Such IBRV antigens are useful to identify antibodies directed against the wild-type IBRV. The virus is also useful as a source of antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

HOMOLOGY VECTOR 848-08. The homology vector 848-08 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and the infectious bovine rhinotracheitis virus (IBRV) glycoprotein C (gC) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will

result. Note that the ß galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the IBRV gC gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 1563 base pair EcoRI/BamHI fragment generated by PCR which contains the coding sequence of the IBRV gC gene. The IBRV gC gene coding region was cloned by PCR using the HindIII I fragment of the IBRV Cooper strain (pSY 830-71) as DNA template and the following PCR primers: 5'-CGGGATCCCTAGGGCGCGGAGCCGAGGGC-3' (4/96.14; **SEQ ID NO 24**) at the 3' end of the IBRV gC gene and combined with 5'- CGGAATTCAGGCCCGCTGGGCGAGCGTGG-3' (4/96.13; **SEQ ID NO 25**) at the 5' end of the IBRV gC gene. The DNA encodes approximately 521 amino acids of the IBRV gC protein. Fragment 5 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique PstI sites using PstI linkers. The NdeI sites in fragments 3 and 5 were converted to unique Not I sites using NotI linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to

2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted which would span SPV fragments 3 and 5.

S-SPV-115

S-SPV-115 is a swinepox virus that expresses two foreign genes. The gene E. coli ß-galactosidase (lacZ) is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV O1L open reading frame). The gene for infectious bovine rhinotracheitis virus (IBRV) glycoprotein B (gB) was inserted into the unique NotI restriction site (NotI linkers inserted into a unique NdeI site in the SPV O1L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted). The lacZ gene is under the control of the synthetic late promoter (LP1), the IBRV gB gene is under the control of the synthetic late/early promoter (LP2EP2). The direction of transcription of the IBRV gB gene is the same as the direction of transcription of the lacZ gene and the SPV O1L gene.

S-SPV-115 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 847-19.59 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 115. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue

plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-115 is useful as a vaccine in bovine against disease caused for infectious bovine rhinotracheitis virus. The IBRV gB antigen is key to raising a protective immune response in the animal. The recombinant viruses are useful alone or in combination as an effective vaccine. S-SPV-115 is also useful as an expression vector for expressing IBRV antigens. Such IBRV antigens are useful to identify antibodies directed against the wild-type IBRV. The virus is also useful as a source of antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

HOMOLOGY VECTOR 847-19.59. The homology vector 847-19.59 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and the infectious bovine rhinotracheitis virus (IBRV) glycoprotein B (gB) gene flanked by SPV DNA. The direction of transcription of the IBRV gB gene is the same as the direction of transcription of the lacZ gene and the SPV O1L gene. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according

to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the IBRV gB gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 2800 base pair EcoRI/BamHI fragment generated by PCR which contains the coding sequence of the IBRV gB gene. The IBRV gB gene coding region was cloned by PCR using the HindIII A fragment of the IBRV Cooper strain (pSY 830-71) as DNA template and the following PCR primers: 5'-CTTCGGATCCTCATGCCCCCCCGACGTCGGCCATC-3' (4/96.15; **SEQ ID NO 26**) at the 3' end of the IBRV gB gene and combined with 5'- TCATGAATTCGGCCGCTCGCGGCGGTGCTGAACGC-3' (4/96.10; **SEQ ID NO 27**) at the 5' end of the IBRV gB gene. The DNA encodes approximately 932 amino acids of the IBRV gB protein. Fragment 5 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique PstI sites using PstI linkers. The NdeI sites in fragments 3 and 5 were

converted to unique Not I sites using NotI linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted which would span SPV fragments 3 and 5.

S-SPV-119

S-SPV-119 is a swinepox virus that expresses three foreign genes. The gene E. coli ß-galactosidase (lacZ) is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The genes for infectious bovine rhinotracheitis virus (IBRV) glycoprotein D (gD) and glycoprotein I (gI) were inserted into the unique HindIII restriction site (HindIII linkers inserted into a unique NdeI site in the SPV 01L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted). The lacZ gene is under the control of the synthetic late promoter (LP1), the IBRV gD and gI genes are each under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-119 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 835-83 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 119. This virus was assayed for ß-galactosidase expression, purity, and insert

stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-119 is useful as a vaccine in bovine against disease caused for infectious bovine rhinotracheitis virus. The IBRV gD and gI antigens are key to raising a protective immune response in the animal. The recombinant viruses are useful alone or in combination as an effective vaccine. S-SPV-119 is also useful as an expression vector for expressing IBRV antigens. Such IBRV antigens are useful to identify antibodies directed against the wild-type IBRV. The virus is also useful as a source of antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

HOMOLOGY VECTOR 835-83. The homology vector 835-83 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and the infectious bovine rhinotracheitis virus (IBRV) glycoprotein D (gD) and glycoprotein I (gI) genes flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV,

a virus containing DNA coding for the foreign genes will result. Note that the ß galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the IBRV gI gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 1320 base pair EcoRI/BamHI fragment generated by PCR which contains the coding sequence of the IBRV gD gene. The IBRV gD gene coding region was cloned by PCR using the HindIII K fragment of the IBRV Cooper strain (pSY 524) as DNA template and the following PCR primers: 5'-CGGGATCCTCACCCGGGCAGCGCGCTGTA-3' (4/96.12; **SEQ ID NO 18**) at the 3' end of the IBRV gD gene and combined with 5'- CGGAATTCACAAGGGCCGACATTGGCC-3' (4/96.11; **SEQ ID NO 19**) at the 5' end of the IBRV gD gene. The DNA encodes approximately 440 amino acids of the IBRV gD protein. Fragment 5 is an approximately 1140 base pair EcoRI/BamHI fragment generated by PCR which contains the coding sequence of the IBRV gI gene. The IBRV gI gene coding region was cloned by PCR using the HindIII K fragment of the IBRV Cooper strain (pSY 524) as DNA template and the following PCR primers: 5'-

ATCGGGATCCCGTTATTCTTCGCTGATGGTGG-3' (4/96.18; **SEQ ID NO 20**) at the 3' end of the IBRV gI gene and combined with 5'-ATCGGAATTCGCGGTGCCTGTTGCTCTGGATG-3' (4/96.17; **SEQ ID NO 21**) at the 5' end of the IBRV gI gene. The DNA encodes approximately 380 amino acids of the IBRV gI protein. Fragment 6 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique PstI sites using PstI linkers. The NdeI sites in fragments 3 and 6 were converted to unique Not I sites using NotI linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted which would span SPV fragments 3 and 6.

Example 42: CANINE CONSTRUCTS AND VACCINES

S-SPV-114

S-SPV-114 is a swinepox virus that expresses two foreign genes. The gene E. coli ß-galactosidase (lacZ) is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The gene for canine parvovirus (CPV) VP2 protein was inserted into the unique NotI restriction site (NotI linkers inserted into a unique NdeI site in the SPV O1L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted). The lacZ gene is under the control of the synthetic late promoter (LP1), the CPV VP2 gene is under the control of the synthetic late/early promoter (LP2EP2). The direction of transcription of the CPV VP2 gene is the same as the direction of transcription of the lacZ gene and the SPV O1L gene.

S-SPV-114 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 848-15.14 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 114. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all

plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-114 was assayed for expression of CPV-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Canine antiserum to CPV (from NVSL) was shown to react specifically with S-SPV-114 plaques and not with S-SPV-003 negative control plaques. All S-SPV-114 observed plaques reacted with the antiserum indicating that the virus was stably expressing the CPV foreign gene.

To confirm the expression of the CPV VP2 gene product, cells were infected with S-SPV-114 and samples of infected cell lysates were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A canine antiserum to CPV (from NVSL) was used to detect expression of CPV specific proteins. The cell lysate from S-SPV-114 infected cells exhibited bands corresponding to 60 kd, which are the expected size of the CPV VP2 protein.

S-SPV-114 is useful as a vaccine in canine against disease caused by canine parvovirus. The CPV VP2 antigen is key to raising a protective immune response in the animal. The recombinant viruses are useful alone or in combination as an effective vaccine. S-SPV-114 is also useful as an expression vector for expressing CPV antigens. Such CPV antigens are useful to identify antibodies directed against the wild-type CPV. The virus is also useful as a source of antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the viral

proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

HOMOLOGY VECTOR 848-15.14. The homology vector 848-15.14 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and for canine parvovirus (CPV) VP2 protein gene flanked by SPV DNA. The direction of transcription of the CPV VP2 gene is the same as the direction of transcription of the lacZ gene and the SPV O1L gene. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the CPV VP2 gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII

M fragment. Fragment 4 is an approximately 1758 base pair EcoRI/BamHI fragment generated by PCR which contains the coding sequence of the CPV VP2 gene. The CPV VP2 gene coding region was cloned by PCR using DNA from CPV 2B field isolate (NVSL) as DNA template and the following PCR primers: 5'-CG<u>GGATCC</u>TTAATATAATTTTCTAGGTGCTAGTTG -3' (4/96.26; **SEQ ID NO 28**) at the 3' end of the CPV VP2 gene and combined with 5'- CG<u>GAATTC</u>GATGAGTGATGGAGCAGTTCAA -3' (4/96.25; **SEQ ID NO 29**) at the 5' end of the CPV VP2 gene. The DNA encodes approximately 586 amino acids of the CPV VP2 protein. Fragment 5 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique PstI sites using PstI linkers. The NdeI sites in fragments 3 and 5 were converted to unique Not I sites using NotI linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted which would span SPV fragments 3 and 5.

<u>S-SPV-116</u>

S-SPV-116 is a swinepox virus that expresses two foreign genes. The gene E. coli ß-galactosidase (lacZ) is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The gene for canine parvovirus (CPV) VP2 protein was inserted into the unique NotI restriction site (NotI linkers inserted into a unique NdeI site in the SPV O1L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted). The lacZ gene is

under the control of the synthetic late promoter (LP1), the CPV VP2 gene is under the control of the synthetic late/early promoter (LP2EP2). The direction of transcription of the CPV VP2 gene is opposite the direction of transcription of the lacZ gene and the SPV O1L gene.

S-SPV-116 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 848-15.13 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 116. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-116 was assayed for expression of CPV-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Canine antiserum to CPV (from NVSL) was shown to react specifically with S-SPV-116 plaques and not with S-SPV-003 negative control plaques. All S-SPV-116 observed plaques reacted with the antiserum indicating that the virus was stably expressing the CPV foreign gene.

To confirm the expression of the CPV VP2 gene product, cells were infected with S-SPV-116 and samples of infected cell lysates were subjected to SDS

polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A canine antiserum to CPV (from NVSL) was used to detect expression of CPV specific proteins. The cell lysate from S-SPV-116 infected cells exhibited bands corresponding to 60 kd, which are the expected size of the CPV VP2 protein.

S-SPV-116 is useful as a vaccine in canine against disease caused by canine parvovirus. The CPV VP2 antigen is key to raising a protective immune response in the animal. The recombinant viruses are useful alone or in combination as an effective vaccine. S-SPV-116 is also useful as an expression vector for expressing CPV antigens. Such CPV antigens are useful to identify antibodies directed against the wild-type CPV. The virus is also useful as a source of antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

HOMOLOGY VECTOR 848-15.13. The homology vector 848-15.13 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and for canine parvovirus (CPV) VP2 protein gene flanked by SPV DNA. The direction of transcription of the CPV VP2 gene is opposite the direction of transcription of the lacZ gene and the SPV O1L gene. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an

approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the CPV VP2 gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 1758 base pair EcoRI/BamHI fragment generated by PCR which contains the coding sequence of the CPV VP2 gene. The CPV VP2 gene coding region was cloned by PCR using DNA from CPV 2B field isolate (NVSL) as DNA template and the following PCR primers: 5'-CG<u>GGATCC</u>TTAATATAATTTTCTAGGTGCTAGTTG -3' (4/96.26; **SEQ ID NO 30**) at the 3' end of the CPV VP2 gene and combined with 5'- CG<u>GAATTC</u>GATGAGTGATGGAGCAGTTCAA -3' (4/96.25; **SEQ ID NO 31**) at the 5' end of the CPV VP2 gene. The DNA encodes approximately 586 amino acids of the CPV VP2 protein. Fragment 5 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique PstI sites using PstI

linkers. The NdeI sites in fragments 3 and 5 were converted to unique Not I sites using NotI linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted which would span SPV fragments 3 and 5.

S-SPV-117

S-SPV-117 is a swinepox virus that expresses two foreign genes. The gene E. coli ß-galactosidase (lacZ) is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The gene for canine parvovirus (CPV) VP1/2 protein was inserted into the unique NotI restriction site (NotI linkers inserted into a unique NdeI site in the SPV 01L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted. The lacZ gene is under the control of the synthetic late promoter (LP1), the CPV VP1/2 gene is under the control of the synthetic late/early promoter (LP2EP2). The direction of transcription of the CPV VP1/2 gene is opposite the direction of transcription of the lacZ gene and the SPV 01L gene.

S-SPV-117 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 848-52A31 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant

virus designated S-SPV 117. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-117 is useful as a vaccine in canine against disease caused by canine parvovirus. The CPV VP1/2 antigen is key to raising a protective immune response in the animal. The recombinant viruses are useful alone or in combination as an effective vaccine. S-SPV-117 is also useful as an expression vector for expressing CPV antigens. Such CPV antigens are useful to identify antibodies directed against the wild-type CPV. The virus is also useful as a source of antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

HOMOLOGY VECTOR 848-52A31. The homology vector 848-52A31 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and for canine parvovirus (CPV) VP1/2 protein gene flanked by SPV DNA. The direction of transcription of the CPV VP1/2 gene is opposite the direction of transcription of the lacZ gene and the SPV O1L gene. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an

approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the CPV VP1/2 gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 2172 base pair EcoRI/BamHI fragment generated by PCR which contains the coding sequence of the CPV VP1/2 gene. The CPV VP1/2 gene coding region was cloned by PCR using DNA from CPV 2B field isolate (NVSL) as DNA template and the following PCR primers: 5'-CGGGATCCTTAATATAATTTTCTAGGTGCTAGTTG -3' (4/96.26; **SEQ ID NO 32**) at the 3' end of the CPV VP1/2 gene and combined with 5'- CGGAATTCTATGTGTTTTTTATAGGACTT -3' (5/96.25; **SEQ ID NO 33**) at the 5' end of the CPV VP1/2 gene. The DNA encodes approximately 724 amino acids of the CPV VP1/2 protein. Fragment 5 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique PstI sites using PstI

linkers. The NdeI sites in fragments 3 and 5 were converted to unique Not I sites using NotI linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted which would span SPV fragments 3 and 5.

S-SPV-118

S-SPV-118 is a swinepox virus that expresses two foreign genes. The gene E. coli ß-galactosidase (lacZ) is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV O1L open reading frame). The gene for canine parvovirus (CPV) VP1/2 protein was inserted into the unique NotI restriction site (NotI linkers inserted into a unique NdeI site in the SPV O1L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted). The lacZ gene is under the control of the synthetic late promoter (LP1), the CPV VP1/2 gene is under the control of the synthetic late/early promoter (LP2EP2). The direction of transcription of the CPV VP1/2 gene is the same as the direction of transcription of the lacZ gene and the SPV O1L gene.

S-SPV-118 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 848-52C8 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final

result of red plaque purification was the recombinant virus designated S-SPV 118. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign gene.

S-SPV-118 is useful as a vaccine in canine against disease caused by canine parvovirus. The CPV VP1/2 antigen is key to raising a protective immune response in the animal. The recombinant viruses are useful alone or in combination as an effective vaccine. S-SPV-118 is also useful as an expression vector for expressing CPV antigens. Such CPV antigens are useful to identify antibodies directed against the wild-type CPV. The virus is also useful as a source of antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

HOMOLOGY VECTOR 848-52C8. The homology vector 848-52C8 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and for canine parvovirus (CPV) VP1/2 protein gene flanked by SPV DNA. The direction of transcription of the CPV VP1/2 gene is the same as the direction of transcription of the lacZ gene and the SPV O1L gene. Upstream of the foreign genes is an approximately 1484 base pair fragment of

SPV DNA. Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the CPV VP1/2 gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 2172 base pair EcoRI/BamHI fragment generated by PCR which contains the coding sequence of the CPV VP1/2 gene. The CPV VP1/2 gene coding region was cloned by PCR using DNA from CPV 2B field isolate (NVSL) as DNA template and the following PCR primers: 5'-CGGGATCCTTAATATAATTTTCTAGGTGCTAGTTG -3' (4/96.26; **SEQ ID NO 34**) at the 3' end of the CPV VP1/2 gene and combined with 5'- CGGAATTCTATGTGTTTTTTATAGGACTT -3' (5/96.25; **SEQ ID NO 35**) at the 5' end of the CPV VP1/2 gene. The DNA encodes approximately 724 amino acids of the CPV VP1/2 protein. Fragment 5 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and

3 were converted to unique PstI sites using PstI linkers. The NdeI sites in fragments 3 and 5 were converted to unique Not I sites using NotI linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted which would span SPV fragments 3 and 5.

Example 43: AVIAN CONSTRUCTS AND VACCINES

S-SPV-105:

S-SPV-105 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* ß-galactosidase (lacZ) and the gene for chicken interferon gamma (cIFNγ) were inserted into the 738-94.4 ORF (a 773 base pair deletion of the SPV 01L ORF; Deletion of nucleotides 1679 to 2452, SEQ ID NO: 189). The lacZ gene is under the control of the swinepox 01L promoter, and the cIFNγ gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-105 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 840-72.A1 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-105. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-105 is confirmed to have cIFNγ activity by measuring the inhibition of vesicular stomatitis virus growth in permissive cells by cocultivation with S-SPV-105 compared to cocultivation of VSV with S-SPV-003.

S-SPV-105 is useful as a vaccine in chickens to stimulate a humoral and cell mediated immune response against infection by avian pathogens. S-SPV-105 is useful for expression of cIFNγ.

HOMOLOGY VECTOR 840-72.A1. The plasmid 840-72.A1 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lac Z) marker gene and an chicken interferon gamma (cIFNγ) gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 855 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1113 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß galactosidase (lacZ) marker gene is under the control of a swinepox virus 01L gene promoter and the cIFNγ gene is under the control of the late/early promoter (LP2EP2). The LP2EP2 cIFNγ gene cassette was inserted into a EcoRI and BamHI site of homology vector 752-22.1. Homology vector 840-72.A1 was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2519 base pair HindIII to SphI restriction fragment of pSP65 (Promega). Fragment 1 is an approximately 855 base pair sub-fragment of the SPV HindIII restriction fragment M (23) synthesized by polymerase chain reaction using DNA primers 5' GAAGCATGCCCGTTCTTATCAATAGTTTAGTCGAAAATA-3 and 5'-CATAAGATCTGGCATTGTGTTATTATACTAACAAAAATAAG-3' to produce an 855 base pair fragment with SphI and BglII ends. Fragment 2 is a 3002 base pair *Bam*HI to *Pvu*II fragment

derived from plasmid pJF751 (49) containing the *E. coli* lacz gene. Fragment 3 is an approximately 522 base pair EcoRI to BglII fragment coding for the cIFNγ gene (62) derived by reverse transcription and polymerase chain reaction (PCR) (Sambrook, et al., 1989) of RNA ISOLATED FROM CONCANAVALIN A STIMULATED CHICKEN SPLEEN CELLS. The antisense primer used for reverse transcription and PCR was 5' CGTCAGATCTCAGGAGGTCATAAGATGCCATTAGC-3' (1/96.38; **SEQ ID NO 36**). The sense primer used for PCR was 5' CGTTGAATTCGATGACTTGCCAGACTTACAACTTG-3' (1/96.37; **SEQ ID NO 37**). The DNA fragment contains the open reading frame of 168 amino acids of the chicken interferon gamma protein. The native methionine codon of cIFNγ is preceded by DNA codons for methionine-asparagine-serine. Fragment 4 is an approximately 1113 base pair subfragment of the SPV HindIII fragment M synthesized by polymerase chain reaction using DNA primers 5'-CCGTAGTCGACAAAGATCGACTTATTAATATGTATGGGATT-3' and 5' GCCTGAAGCTTCTAGTACAGTATTTACGACTTTTGAAAT-3' to produce an 1113 base pair fragment with SalI and HindIII ends.

S-SPV-086

S-SPV-086 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli B-galactosidase (lacZ) and antisense of the gene for chicken interferon gamma (cIFNγ) were inserted into the 738-94.4 ORF (a 773 base pair deletion of the SPV OIL ORF; Deletion of nucleotides 1679 to 2452, SEQ ID NO: 189), The lacZ gene is under the control of the swinepox OIL promoter, and the antisense-cIFNγ gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-086 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 836-62.B1 and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING B-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-086. This virus was assayed for B-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene. Homology vector 836-62.B1 is constructed in the same manner as homology vector 840-72.A1 except that in 836-62.B1, the approximately 522 base pair EcoRI to BgIII fragment coding for he cIFNγ gene is in the opposite orientation relative to the LP2EP2 promoter compared to 840-72.A1.

S-SPV-086 was assayed for expression of B-galactosidase antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Anti-B-galactosidase antiserum was shown to react specifically with S-SPV-086 plaques and not with S-SPV-003 negative control plaques. All S-SPV-086 observed plaques reacted with the antiserum indicating that the virus was stably expressing the B-galactosidase foreign gene.

S-SPV-086 is useful for expression of antisense RNA to the cIFNγ mRNA. When S-SPV-986 is transfected into duck embryo fibroblasts (DEF) or chicken embryo fibroblast (CEF) cells, it will not lyse the cells, but will express antisense cIFNγ RNA in CEF cells and inhibit expression of cIFNγ protein from DEF or CEF cells. Recombinant viruses, such as herpesvirus of turkeys

(HVT) or Marek's disease virus, grow to higher titers (10^8 to 10^{10} pfu/ml) in S-SPV-086 transfected into DEF or CEF cells, and transfected cells are selected for puromycin resistance by growth in the presence of puromycin. These transfected cells will grow continuously, express antisense to cIFNγ gene and permit high titer growth of herpesvirus to turkeys or Marek's disease virus (10^8 to 10^{10} pfu/ml).

Example 44: FELINE CONSTRUCTS AND VACCINES

S-SPV-106

S-SPV-106 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli ß-galactosidase (lacZ) and the genes for feline immunodeficiency virus (FIV) envelope (env) and gag-protease were inserted into the SPV 617 48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the FIV env and gag-protease genes are each under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-106 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 836-22.A1 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 106. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue

plaque assay as described in Materials and Methods. After the initial five rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-106 was assayed for expression of FIV-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Monoclonal antibodies to FIV gag-protease were shown to react specifically with S-SPV-106 plaques and not with S-SPV-003 negative control plaques. All S-SPV-106 observed plaques reacted with the antiserum indicating that the virus was stably expressing the FIV gag foreign gene.

S-SPV-106 is a recombinant swinepox virus expressing both the FIV env and gag-protease proteins and is useful as a vaccine in cats against FIV infection S-SPV-106 is also useful for expression of the FIV env and gag-protease proteins.

HOMOLOGY VECTOR 836-22.A1. The plasmid 836-22.A1 was used to insert foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and the feline immunodeficiency virus (FIV) envelope (env) and gag/protease genes flanked by SPV DNA. When this plasmid was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the ß galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1) and the FIV env and gag/protease genes are under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the

following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII fragment M (23). Fragment 2 is an approximately 2564 base pair *Bam*HI to *Bam*HI fragment of the FIV *env* gene (61) (approximately 860 amino acids which includes the full length SU and TM coding regions of FIV *env*) synthesized by CLONING WITH THE POLYMERASE CHAIN REACTION. The template for the PCR reaction was FIV strain PPR genomic cDNA (61). The upstream primer 10/93.21 (5'-GCCCGGATCCTATGCAGAAGGGTTTGCAGC-3';) was synthesized corresponding to the 5' end of the FIV *env* gene starting at nucleotide 6263 of FIV strain PPR genomic cDNA, and the procedure introduced a *Bam*HI site at the 5' end. The downstream primer 10/93.20 (5'-CCGTGGATCCGGCACTCCATCATTCCTCCTC-3';) was synthesized corresponding to the 3' end of the FIV env gene starting at nucleotide 8827 of FIV PPR genomic cDNA. Fragment 3 is an approximately 1878 base pair EcoRI to BglII restriction fragment of the FIV gag/protease (gag ORF is approximately 452 amino acids; protease ORF is approximately 209 amino acids) synthesized by polymerase chain reaction (PCR) using cDNA from the FIV (PPR strain) (61). The primer (5' GCGTGAATTCGGGGAATGGACAGGGGCGAGAT-3'; 11/94.9) synthesizes from the 5' end of the FIV gag-protease gene, introduces an EcoRI site at the 5' end of the gene. The primer (5'-GAGCCAGATCTGCTCTTTTTACTTTCCC-3'; 11/94.10) synthesizes from the 3' end of the FIV gag-protease gene and introduces a BglII site at the 3' end of the gene. The PCR product was digested with EcoRI and BglII to yield a fragment 1878 base pairs in length

corresponding to the FIV gag-protease gene. Fragment 4 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 5 is an approximately 2149 base pair AccI to HindIII restriction sub-fragment of the SPV HindIII restriction fragment M (23). The AccI site in the SPV homology vector was converted to a unique NotI site.

S-SPV-127

S-SPV-127 is a swinepox virus that expresses four foreign genes. The gene for E. coli ß-galactosidase (lacZ) and the gene for the feline immunodeficiency virus (FIV) gag/protease (gag) were inserted into the 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of nucleotides 1669 to 2452, SEQ ID NO: 189). The gene for E. coli ß-glucuronidase (uidA) and the gene for the feline immunodeficiency virus (FIV) envelope (env) were inserted into a unique NotI site (NotI linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region (SEQ ID NO 1) of the 6.7 kb SPV HindIII K fragment). The lacZ gene is under the control of the swinepox O1L promoter, the uidA gene is under the control of the synthetic early promoter (EP2) and the FIV gag/protease and envelope genes are each under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-127 was derived from S-SPV-046 (Kasza Strain). This was accomplished utilizing the homology vector 849-61.A14 (see Materials and Methods) and virus S-SPV-046 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase and ß-glucuronidase (BLUOGAL AND CPRG

ASSAYS and SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of red plaque and blue plaque purification was the recombinant virus designated S-SPV-127. This virus was assayed for ß-galactosidase and ß-glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign genes.

S-SPV-127 is useful as a vaccine in cats against FIV infection S-SPV-127 is also useful for expression of the FIV env and gag proteins.

HOMOLOGY VECTOR 849-61.A14. The plasmid 849-61.A14 was used to insert foreign DNA into SPV. It incorporates an E. coli ß-glucuronidase (uidA) marker gene and the feline immunodeficiency virus (FIV) envelope (env) gene flanked by SPV DNA. When this plasmid was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the ß-glucuronidase (uidA) gene is under the control of a synthetic early pox promoter (EP2) and the FIV env gene is under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 3005 base pair *Hin*dIII restriction fragment of pSP65 (Promega). Fragment 1 is an approximately 1652 base pair *Hin*dIII to *Eco*RI

restriction sub-fragment of the SPV *Hin*dIII restriction fragment K. Fragment 2 is an approximately 2564 base pair *Bam*HI to *Bam*HI fragment of the FIV *env* gene (61) (approximately 860 amino acids which includes the full length SU and TM coding regions of FIV *env*) synthesized by CLONING WITH THE POLYMERASE CHAIN REACTION. The template for the PCR reaction was FIV strain PPR genomic cDNA (61). The upstream primer 10/93.21 (5'-GCCCGGATCCTATGGCAGAAGGGTTTGCAGC-3';) was synthesized corresponding to the 5' end of the FIV *env* gene starting at nucleotide 6263 of FIV strain PPR genomic cDNA, and the procedure introduced a *Bam*HI site at the 5' end. The downstream primer 10/93.20 (5'-CCGTGGATCCGGCACTCCATCATTCCTCCTC-3';) was synthesized corresponding to the 3' end of the FIV env gene starting at nucleotide 8827 of FIV PPR genomic cDNA, and the procedure introduced a *BamHI* site at the 3' end. Fragment 3 is an approximately 1800 base pair *Eco*RI to *Xma*I restriction fragment containing the *E. coli uidA* gene. Fragment 4 is an approximately 3010 base pair *Bam*HI to *Pvu*II restriction fragment of plasmid pJF751 (11). Fragment 5 is an approximately 5053 base pair *Eco*RI to *Hin*dIII restriction sub-fragment of the SPV *Hin*dIII restriction fragment K. The *Eco*RI site in fragments 1 and 5 of the SPV homology vector was converted to a unique *Not*I site.

<u>S-SPV-089</u>

S-SPV-089 is a swinepox virus that expresses three foreign genes. . The gene for *E. coli* ß-galactosidase (lacZ) and the gene for feline leukemia virus (FeLV) gag/protease were inserted into the 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of

nucleotides 1679 to 2452, SEQ ID NO: 189). The lacZ gene is under the control of the swinepox O1L promoter, and the FeLV gag/protease gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-089 is derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the HOMOLOGY VECTOR 832-26.A1 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 089. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus is pure, stable, and expressing the foreign gene.

S-SPV-089 was assayed for expression of FeLV-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal mouse anti-p27 serum, rabbit anti-p27 serum and rabbit anti-ß-galactosidase were shown to react specifically with S-SPV-089 plaques and not with S-SPV-003 negative control plaques. All S-SPV-089 observed plaques reacted with the antiserum indicating that the virus was stably expressing the FeLV gag protease and E. coli ß-galactosidase proteins.

S-SPV-089 is useful as a vaccine in cats against disease caused by feline leukemia virus. The FeLV gag/protease antigen is key to raising a protective

immune response in the animal. The recombinant viruses are useful alone or in combination as an effective vaccine. S-SPV-089 is also useful as an expression vector for expressing FeLV antigens. Such FeLV antigens are useful to identify antibodies directed against the wild-type FeLV. The virus is also useful as a source of antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

HOMOLOGY VECTOR 832-26.A1. The plasmid 832-26.A1 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lac Z) marker gene and the feline leukemia virus (FeLV) gag/protease gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 855 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1113 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß galactosidase (lacZ) marker gene is under the control of a swinepox virus 01L gene promoter and the FeLV gag/protease gene is under the control of the late/early promoter (LP2EP2). The LP2EP2 FeLV gag/protease gene cassette was inserted into a EcoRI and BamHI site of homology vector 752-22.1. Homology vector 832-26.A1 was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid

vector was derived from an approximately 2519 base pair HindIII to SphI restriction fragment of pSP65 (Promega). Fragment 1 is an approximately 855 base pair sub-fragment of the SPV HindIII restriction fragment M (23) synthesized by polymerase chain reaction using DNA primers 5' GAAGCATGCCCGTTCTTATCAATAGTTTAGTCGAAAATA-3' and 5'-CATAAGATCTGGCATTGTGTTATTATACTAACAAAAATAAG-3' to produce an 855 base pair fragment with SphI and BglII ends. Fragment 2 is a 3002 base pair *Bam*HI to *Pvu*II fragment derived from plasmid pJF751 (49) containing the *E. coli* lacZ gene. Fragment 3 is an approximately 2160 base pair EcoRI to BamHI restriction fragment of the FeLV gag/protease (gag ORF is approximately 584 amino acids; protease ORF is approximately 136 amino acids) synthesized by polymerase chain reaction (PCR) using cDNA from FeLV/FAIDS strain, Type A (cDNA clone p61E; Dr. Mullens, NIAIDS repository). The primer (5' CGTC<u>GAATTC</u>GATGTCTGGAGCCTCTAGTGGGA-3'; 1/96.32) (**SEQ ID NO 38**) synthesizes from the 5' end of the FeLV gag/protease gene, introduces an EcoRI site at the 5' end of the gene and an ATG start codon. The primer (5'- CGTC<u>GGATCC</u>GGCTCAAATAGCCGATACTCTTCTT-3'; 1/96.33) (**SEQ ID NO 39**) synthesizes from the 3' end of the FeLV gag/protease gene. The PCR product was digested with EcoRI and BglII to yield a fragment 2160 base pairs in length corresponding to the FeLV gag/protease gene. Fragment 4 is an approximately 1113 base pair subfragment of the SPV HindIII fragment M synthesized by polymerase chain reaction using DNA primers 5'-CCGTAGTCGACAAAGATCGACTTATTAATATGTATGGGATT-3' and 5' GCCTGAAGCTTCTAGTACAGTATTTACGACTTTTGAAAT-3' to produce an 1113 base pair fragment with SalI and HindIII ends.

S-SPV-100

S-SPV-100 is a swinepox virus that expresses two foreign genes. The gene for E. coli ß-galactosidase (lacZ) and the gene for feline leukemia virus (FeLV) envelope (env) gp70 + p15E were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the FeLV env gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-100 is derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the HOMOLOGY VECTOR 843-9.322 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-100. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus is pure, stable, and expressing the foreign gene.

S-SPV-100 was assayed for expression of FeLV-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal mouse anti-p70 serum and rabbit anti-ß-galactosidase were shown to react specifically with S-SPV-100 plaques and not with S-SPV-003 negative control plaques. All S-SPV-100 observed plaques reacted with the antiserum indicating

that the virus was stably expressing the FeLV env and E. coli ß-galactosidase proteins.

S-SPV-100 is useful as a vaccine in cats against disease caused by feline leukemia virus. The FeLV env antigen is key to raising a protective immune response in the animal. The recombinant viruses are useful alone or in combination as an effective vaccine. S-SPV-100 is also useful as an expression vector for expressing FeLV antigens. Such FeLV antigens are useful to identify antibodies directed against the wild-type FeLV. The virus is also useful as a source of antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

HOMOLOGY VECTOR 843-9.322. The plasmid 843-9.322 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and a feline leukemia virus (FeLV) envelope (env) gp70 + p15E gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß-galactosidase (lacZ) marker gene is under the control of a late promoter (LP1) and the FeLV env gene is under the control of the late/early promoter (LP2EP2). It was

constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 1973 base pair EcoRI to BamHI restriction fragment of the FeLV env (gp70 + p15E) (env ORF is approximately 658 amino acids) synthesized by polymerase chain reaction (PCR) using cDNA from FeLV/FAIDS strain, Type A (cDNA clone p61E; Dr. Mullens, NIAIDS repository) The primer (5-'CGTCGAATTCAATGGAAAGTCCAACGCACCCAAAA-3'; 1/96.31) (**SEQ ID NO 40**) synthesizes from the 5' end of the FeLV env gene, introduces an EcoRI site at the 5' end of the gene and an ATG start codon. The primer (5'-CGTCGGATCCGGGGACTAAATGGAATCATACA -3'; 1/96.28) (**SEQ ID NO 41**) synthesizes from the 3' end of the FeLV env gene. The PCR product was digested with EcoRI and BglII to yield a fragment 1973 base pairs in length corresponding to the FeLV env gene. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 2149 base pair AccI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique NotI sites using NotI linkers.

S-SPV-107 and S-SPV-108

S-SPV-107 is a swinepox virus that expresses three foreign genes. The gene E. coli ß-galactosidase (lacZ)

is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The gene for feline leukemia virus (FeLV) gag/protease and envelope (env) gp70 + p15E was inserted into the unique NotI restriction site (NotI linkers inserted into a unique NdeI site in the SPV O1L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted). The lacZ gene is under the control of the synthetic late promoter (LP1), the FeLV gag/protease and env genes are under the control of the synthetic late/early promoter (LP2EP2). The direction of transcription of the FeLV gag/protease and env gene are the same as direction of transcription of the lacZ gene and the SPV O1L gene.

S-SPV-107 is derived from S-SPV-001 (Kasza Strain). This is accomplished utilizing the HOMOLOGY VECTOR 840-68.A1 and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock is screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification is the recombinant virus designated S-SPV 107. This virus is assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus is pure, stable, and expressing the foreign gene.

S-SPV-108 is a swinepox virus that expresses three foreign genes. The gene E. coli ß-galactosidase (lacZ)

is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The gene for feline leukemia virus (FeLV) gag/protease and envelope (env) gp70 + p15E was inserted into the unique NotI restriction site (NotI linkers inserted into a unique NdeI site in the SPV O1L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted). The lacZ gene is under the control of the synthetic late promoter (LP1), the FeLV gag/protease and env gene is under the control of the synthetic late/early promoter (LP2EP2). The direction of transcription of the FeLV gag/protease and env gene are opposite the direction of transcription of the lacZ gene and the SPV O1L gene.

S-SPV-108 is derived from S-SPV-001 (Kasza Strain). This is accomplished utilizing the HOMOLOGY VECTOR VECTOR 840-68.A6 and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock is screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification is the recombinant virus designated S-SPV-108. This virus is assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus is pure, stable, and expressing the foreign gene.

S-SPV-107 or S-SPV-108 are useful as a vaccine in cats against disease caused by feline leukemia virus. The

FeLV gag/protease and env antigens are key to raising a protective immune response in the animal. The recombinant viruses are useful alone or in combination as an effective vaccine. S-SPV-107 or S-SPV-108 are also useful as an expression vector for expressing FeLV antigens. Such FeLV antigens are useful to identify antibodies directed against the wild-type FeLV. The virus is also useful as a source of antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

HOMOLOGY VECTORS 840-68.A1 AND 840-68.A6. The homology vector are constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and for feline leukemia virus (FeLV) protease (gag) and envelope (env) gp70 + p15E genes flanked by SPV DNA. The direction of transcription of the FeLV gag/protease and env gene is the same as direction of transcription of the LacZ gene and the SPV O1L gene in homology vector 840-68.A1. The direction of transcription of the FeLV gag/protease and env gene is opposite the direction of transcription of the lacZ gene and the SPV O1L gene in 840-68.A6. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the

ß galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the FeLV gag/protease and env gene are each under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 2160 base pair EcoRI to BamHI restriction fragment of the FeLV gag/protease (gag ORF is approximately 584 amino acids; protease ORF is approximately 136 amino acids) synthesized by polymerase chain reaction (PCR) using cDNA from FeLV/FAIDS strain, Type A (cDNA clone p61E; Dr. Mullens, NIAIDS repository) The primer (5' CGTC<u>GAATTC</u>GATGTCTGGAGCCTCTAGTGGGA-3'; 1/96.32) (**SEQ ID NO 38**) synthesizes from the 5' end of the FeLV gag/protease gene, introduces an EcoRI site at the 5' end of the gene. One in frame start codon (ATG) is in the LP2EP2 promoter, and a second in frame start codon is in the gene coded by the PCR primer. The primer (5'-CGTC<u>GGATCC</u>GGCTCAAATAGCCGATACTCTTCTT-3'; 1/96.33) (**SEQ ID NO 39**) synthesizes from the 3' end of the FeLV gag/protease gene. The PCR product was digested with EcoRI and BglII to yield a fragment 2160 base pairs in length corresponding to the FeLV gag/protease gene.

Fragment 5 is an approximately 1973 base pair EcoRI to BamHI restriction fragment of the FeLV env (gp70 + p15E) (env ORF is approximately 658 amino acids) synthesized by polymerase chain reaction (PCR) using cDNA from FeLV/FAIDS strain, Type A (cDNA clone p61E; Dr. Mullens, NIAIDS repository) The primer (5-'CGTCGAATTCAATGGAAAGTCCAACGCACCCAAAA-3'; 1/96.31) (**SEQ ID NO 40**) synthesizes from the 5' end of the FeLV env gene, introduces an EcoRI site at the 5' end of the gene. One in frame start codon (ATG) is in the LP2EP2 promoter, and a second in frame start codon is in the gene coded by the PCR primer The primer (5'-CGTCGGATCCGGGGACTAAATGGAATCATACA -3'; 1/96.28) (**SEQ ID NO 41**) synthesizes from the 3' end of the FeLV env gene. The PCR product was digested with EcoRI and BglII to yield a fragment 1973 base pairs in length corresponding to the FeLV env gene. Fragment 6 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique PstI sites using PstI linkers. The NdeI sites in fragments 3 and 6 were converted to unique Not I sites using NotI linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted which would span SPV fragments 3 and 6.

S-SPV-128:

S-SPV-128 is a swinepox virus that expresses four foreign genes. The gene for E. coli ß-galactosidase (lacZ) and the gene for for feline leukemia virus (FeLV) gag/protease and were inserted into the 738-94.4

ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of nucleotides 1669 to 2452, SEQ ID NO: 189). The gene for E. coli ß-glucuronidase (uidA) and the gene for the feline leukemia virus (FeLV) envelope (env) gp70 + p15E were inserted into a unique NotI site (NotI linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region (SEQ ID NO 1) of the 6.7 kb SPV HindIII K fragment). The lacZ gene is under the control of the synthetic late promoter (LP1), the uidA gene is under the control of the synthetic early promoter (EP2) and the FeLV gag/protease and envelope genes are each under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-128 was derived from S-SPV-089 (Kasza Strain). This was accomplished utilizing the homology vector 860-2 (see Materials and Methods) and virus S-SPV-089 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase and ß-glucuronidase (BLUOGAL AND CPRG ASSAYS and SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of red plaque and blue plaque purification was the recombinant virus designated S-SPV-128. This virus was assayed for ß-galactosidase and ß-glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign genes.

S-SPV-128 is useful as a vaccine in cats against FIV infection S-SPV-128 is also useful for expression of the FIV env and gag proteins.

HOMOLOGY VECTOR 860-2. The plasmid 860-2 was used to insert foreign DNA into SPV. It incorporates a gene for E. coli ß-glucuronidase (uidA) and the gene for the feline leukemia virus (FeLV) envelope (env) gp70 + p15E flanked by SPV DNA. When this plasmid was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the ß-glucuronidase (uidA) gene is under the control of a synthetic early pox promoter (EP2) and the FeLV env gene is under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 3005 base pair *Hin*dIII restriction fragment of pSP65 (Promega). Fragment 1 is an approximately 1652 base pair *Hin*dIII to *Eco*RI restriction sub-fragment of the SPV *Hin*dIII restriction fragment K. Fragment 2 is an approximately 1973 base pair EcoRI to BamHI restriction fragment of the FeLV env (gp70 + p15E) (approximately 860 amino acids which includes the full length SU and TM coding regions of FIV *env*) synthesized by polymerase chain reaction (PCR) using cDNA from FeLV/FAIDS strain, Type A (cDNA clone p61E; Dr. Mullens, NIAIDS repository) The primer (5'-CGTC<u>GAATTC</u>AATGGAAAGTCCAACGCACCCAAAA-3'; 1/96.31) (**SEQ ID NO 40**) synthesizes from the 5' end of the FeLV env gene, introduces an EcoRI site at the 5' end of the

gene and an ATG start codon. The primer (5'-CGTCGGATCCGGGGACTAAATGGAATCATACA -3'; 1/96.28) **(SEQ ID NO 41)** synthesizes from the 3' end of the FeLV env gene. The PCR product was digested with EcoRI and BglII to yield a fragment 1973 base pairs in length corresponding to the FeLV env gene.. Fragment 3 is an approximately 1800 base pair *Eco*RI to *Xma*I restriction fragment containing the *E. coli uidA* gene. Fragment 4 is an approximately 5053 base pair *Eco*RI to *Hin*dIII restriction sub-fragment of the SPV *Hin*dIII restriction fragment K. The *Eco*RI sites in fragments 1 and 4 of the SPV homology vector were converted to a unique *Not*I site.

PORCINE CONSTRUCTS AND VACCINES

S-SPV-084

S-SPV-084 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli ß-galactosidase (lacZ) and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF6 were inserted into the SPV 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of nucleotides 1669 to 2452, SEQ ID NO: 189). The lacZ gene is under the control of the swinepox POIL promoter and the PRRS ORF6 gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-084 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 831-38.22 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 084. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

To confirm the expression of the PRRS ORF6 matrix protein gene product, cells were infected with S-SPV-084 and samples of infected cell lysates were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING

PROCEDURE. A polyclonal swine anti-PRRS (NVSL) serum was used to detect expression of PRRS specific proteins. The cell lysate from S-SPV-084 infected cells exhibited a band corresponding to 18 kd, which is the expected size of the PRRS ORF6 matrix protein.

S-SPV-084 is useful as a vaccine in swine against PRRS infection S-SPV-084 is also useful for expression of the PRRS ORF6 matrix protein.

HOMOLOGY VECTOR 831-38.22. The homology vector 831-38.22 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and the porcine reproductive and respiratory syndrome virus (PRRS) ORF6 matrix gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 855 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1113 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß galactosidase (lacZ) marker gene is under the control of the swinepox virus O1L promoter and the PRRS ORF6 matrix gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2519 base pair HindIII to SphI restriction fragment of pSP65 (Promega). Fragment 1 is an approximately 855 base pair sub-fragment of the SPV HindIII restriction fragment M (23) synthesized by polymerase chain reaction using DNA primers 5' GAAGCATGCCCGTTCTTATCAATAGTTTAGTCGAAAATA-3' and 5'-CATAAGATCTGGCATTGTGTTATTATACTAACAAAAATAAG-3' to produce

an 855 base pair fragment with SphI and BglII ends. Fragment 2 is an approximately 3002 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an EcoRI to BamHI restriction fragment synthesized by reverse transcription and polymerase chain reaction (PCR) using genomic RNA from a U.S. Isolate of PRRS obtained from the NVSL (Reference strain, IA-2). To synthesize PRRS ORF6 matrix gene, the primer (5' CGGGAATTCGGGGTCGTCCTTAGATGACTTCTGCC-3'; 1/96.17) **(SEQ ID NO 42)** synthesizes from the 5' end of the PRRS ORF6 gene, introduces an EcoRI site at the 5' end of the gene. The primer (5' GCGGATCCTTGTTATGTGGCATATTTGACAAGGTTTAC-3'; 1/96.18) **(SEQ ID NO 43)** is used for reverse transcription and PCR and synthesizes from the 3' end of the PRRS ORF6 gene. The PCR product was digested with EcoRI and BamHI to yield a fragment 532 base pairs in length corresponding to the PRRS ORF6 gene. Fragment 4 is an approximately 1113 base pair subfragment of the SPV HindIII fragment M synthesized by polymerase chain reaction using DNA primers 5'-CCGTAGTCGACAAAGATCGACTTATTAATATGTATGGGATT-3' and 5' GCCTGAAGCTTCTAGTACAGTATTTACGACTTTTGAAAT-3' to produce an 1113 base pair fragment with SalI and HindIII ends.

S-SPV-091

S-SPV-091 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli ß-galactosidase (lacZ) and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF2 were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the PRRS ORF2 gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-091 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 844-15.110 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 091. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-091 is useful as a vaccine in swine against PRRS infection. S-SPV-091 is also useful for expression of the PRRS ORF2 protein.

HOMOLOGY VECTOR 844-15.110. The plasmid 844-15.110 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and a porcine reproductive and respiratory syndrome virus (PRRS) ORF2 gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß-galactosidase (lacZ) marker gene is under the control of a late promoter (LP1) and the PRRS ORF2 gene is under the control of the late/early promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30),

by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an EcoRI to BamHI restriction fragment synthesized by reverse transcription and polymerase chain reaction (PCR) using genomic RNA from a U.S. Isolate of PRRS obtained from the NVSL (Reference strain, IA-2). To synthesize PRRS ORF2, the primer (5' AATGAATTCGAAATGGGGTCCATGCAAAGCCTTTTTG-3'; 1/96.15) (**SEQ ID NO 44**) synthesized from the 5' end of the PRRS ORF2 gene, introduced an EcoRI site at the 5' end of the gene. The primer (5'-CAAGGATCCCACACCGTGTAATTCACTGTGAGTTCG-3'; 1/96.16) (**SEQ ID NO 45**) was used for reverse transcription and PCR and synthesized from the 3' end of the PRRS ORF2 gene. The PCR product was digested with EcoRI and BamHI to yield a fragment approximately 788 base pairs in length corresponding to the PRRS ORF2 gene. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair AccI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 4 were converted to unique NotI sites using NotI linkers.

S-SPV-092

S-SPV-092 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli ß-galactosidase (lacZ) and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF7 nucleocapsid were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction

site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the PRRS ORF7 nucleocapsid gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-092 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 844-19.94 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 092. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-092 was assayed for expression of PRRS-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-PRRS (NVSL) serum and a monoclonal antibody to PRRS ORF7 nucleocapsid protein were each shown to react specifically with S-SPV-092 plaques and not with S-SPV-003 negative control plaques. All S-SPV-092 observed plaques reacted with the antiserum indicating that the virus was stably expressing the PRRS ORF7 nucleocapsid protein.

S-SPV-092 is useful as a vaccine in swine against PRRS infection. S-SPV-092 is also useful for expression of the PRRS ORF7 nucleocapsid protein.

HOMOLOGY VECTOR 844-19.94. The plasmid 844-19.94 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and a porcine reproductive and respiratory syndrome virus (PRRS) ORF7 gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß-galactosidase (lacZ) marker gene is under the control of a late promoter (LP1) and the PRRS ORF7 gene is under the control of the late/early promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an EcoRI to BamHI restriction fragment synthesized by reverse transcription and polymerase chain reaction (PCR) using genomic RNA from a U.S. Isolate of PRRS obtained from the NVSL (Reference strain, IA-2). To synthesize PRRS ORF7, the primer (5' GTC<u>GAATTC</u>GCCAAATAACAACGGCAAGCAGCAGAAG 3'; 1/96.19) (**SEQ ID NO 46**) synthesized from the 5' end of the PRRS ORF7 gene, introduced an EcoRI site at the 5' end of the gene. The primer (5'-CAA<u>GGATCC</u>CAGCCCATCATGCTGAGGGTGATG-3'; 1/96.20) (**SEQ ID NO 47**) was used for reverse transcription and PCR and synthesized from the 3' end of the PRRS ORF7 gene. The PCR product was digested with EcoRI and BamHI to yield

a fragment approximately 383 base pairs in length corresponding to the PRRS ORF7 gene. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair AccI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 4 were converted to unique NotI sites using NotI linkers.

S-SPV-093

S-SPV-093 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli ß-galactosidase (lacZ) and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF3 were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the PRRS ORF3 gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-093 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 839-58.9 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 093. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-093 was assayed for expression of PRRS-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-PRRS (NVSL) serum was shown to react specifically with S-SPV-093 plaques and not with S-SPV-003 negative control plaques. All S-SPV-093 observed plaques reacted with the antiserum indicating that the virus was stably expressing the PRRS ORF3 protein.

To confirm the expression of the PRRS ORF3 protein gene product, cells were infected with S-SPV-093 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal swine anti-PRRS (NVSL) serum was used to detect expression of PRRS specific proteins. The cell lysate a nd culture supernatant from S-SPV-093 infected cells exhibited a band corresponding to 45 kd, which is the expected size of the PRRS ORF3 protein. ORF3 protein was shown to be secreted from infected cells into the culture media.

S-SPV-093 is useful as a vaccine in swine against PRRS infection. S-SPV-093 is also useful for expression of the PRRS ORF3 protein.

HOMOLOGY VECTOR 839-58.9. The plasmid 839-58.9 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and a porcine reproductive and respiratory syndrome virus (PRRS) ORF3 gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus

containing DNA coding for the foreign genes will result. Note that the ß-galactosidase (lacZ) marker gene is under the control of a late promoter (LP1) and the PRRS ORF3 gene is under the control of the late/early promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an EcoRI to BamHI restriction fragment synthesized by reverse transcription and polymerase chain reaction (PCR) using genomic RNA from a U.S. Isolate of PRRS obtained from the NVSL (Reference strain, IA-2). To synthesize PRRS ORF3, the primer 5'-TTC<u>GAATTC</u>GGCTAATAGCTGTACATTCCTCCATATTT-3'; 1/96.7) **(SEQ ID NO 48)** synthesized from the 5' end of the PRRS ORF3 gene, introduced an EcoRI site at the 5' end of the gene. The primer (5'-GG<u>GGATCC</u>TATCGCCGTACGGCACTGAGGG-3'; 1/96.8) **(SEQ ID NO 49)** was used for reverse transcription and PCR and synthesized from the 3' end of the PRRS ORF3 gene. The PCR product was digested with EcoRI and BamHI to yield a fragment approximately 768 base pairs in length corresponding to the PRRS ORF3 gene. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair AccI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 4 were converted to unique NotI sites using NotI linkers.

<u>S-SPV-094</u>

S-SPV-094 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli ß-galactosidase (lacZ) and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF4 were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the PRRS ORF4 gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-094 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 839-58.36 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 094. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-094 was assayed for expression of PRRS-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-PRRS (NVSL) serum was shown to react specifically with S-SPV-094 plaques and not with S-SPV-003 negative control plaques. All S-SPV-094 observed plaques reacted with the antiserum indicating that the virus was stably expressing the PRRS ORF4 protein.

To confirm the expression of the PRRS ORF4 protein gene product, cells were infected with S-SPV-094 and samples

of infected cell lysates were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal swine anti-PRRS (NVSL) serum was used to detect expression of PRRS specific proteins. The cell lysate from S-SPV-094 infected cells exhibited a band corresponding to 31 kd, which is the expected size of the PRRS ORF4 protein (202 amino acids).

S-SPV-094 is useful as a vaccine in swine against PRRS infection. S-SPV-094 is also useful for expression of the PRRS ORF4 protein.

HOMOLOGY VECTOR 839-58.36. The plasmid 839-58.36 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and a porcine reproductive and respiratory syndrome virus (PRRS) ORF4 gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß-galactosidase (lacZ) marker gene is under the control of a late promoter (LP1) and the PRRS ORF4 gene is under the control of the late/early promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an

EcoRI to BamHI restriction fragment synthesized by reverse transcription and polymerase chain reaction (PCR) using genomic RNA from a U.S. Isolate of PRRS obtained from the NVSL (Reference strain, IA-2). To synthesize PRRS ORF4, the primer (5'-CCGAATTCGGCTGCGTCCCTTCTTTTCCTCATGG-3'; 1/96.11) (**SEQ ID NO 50**) synthesized from the 5' end of the PRRS ORF4 gene, introduced an EcoRI site at the 5' end of the gene. The primer (5' CTGGATCCTTCAAATTGCCAACAGAATGGCAAAAAGAC-3'; 1/96.12) (**SEQ ID NO 51**) was used for reverse transcription and PCR and synthesized from the 3' end of the PRRS ORF4 gene. The PCR product was digested with EcoRI and BamHI to yield a fragment approximately 542 base pairs in length corresponding to the PRRS ORF4 gene. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair AccI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 4 were converted to unique NotI sites using NotI linkers.

S-SPV-095

S-SPV-095 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli ß-galactosidase (lacZ) and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF5 were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the PRRS ORF5 gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-095 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 839-58.43 (see Materials and Methods) and virus S-SPV-

001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 095. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

To confirm the expression of the PRRS ORF5 protein gene product, cells were infected with S-SPV-095 and samples of infected cell lysates were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal swine anti-PRRS (NVSL) serum was used to detect expression of PRRS specific proteins. The cell lysate from S-SPV-095 infected cells exhibited a band corresponding to 26 kd, which is the expected size of the PRRS ORF5 protein.

S-SPV-095 is useful as a vaccine in swine against PRRS infection. S-SPV-095 is also useful for expression of the PRRS ORF5 protein.

HOMOLOGY VECTOR 839-58.43. The plasmid 839-58.43 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and a porcine reproductive and respiratory syndrome virus (PRRS) ORF5 gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is

used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß-galactosidase (lacZ) marker gene is under the control of a late promoter (LP1) and the PRRS ORF5 gene is under the control of the late/early promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an EcoRI to BamHI restriction fragment synthesized by reverse transcription and polymerase chain reaction (PCR) using genomic RNA from a U.S. Isolate of PRRS obtained from the NVSL (Reference strain, IA-2). To synthesize PRRS ORF5, the primer (5'-TTGAATTCGTTGGAGAAATGCTTGACCGCGGGC-3'; 1/96.13) **(SEQ ID NO 52**(synthesized from the 5' end of the PRRS ORF5 gene, introduced an EcoRI site at the 5' end of the gene. The primer (5'-GAAGGATCCTAAGGACGACCCCATTGTTCCGCTG-3'; 1/96.14) **(SEQ ID NO 53**) was used for reverse transcription and PCR and synthesized from the 3' end of the PRRS ORF5 gene. The PCR product was digested with EcoRI and BamHI to yield a fragment approximately 606 base pairs in length corresponding to the PRRS ORF5 gene. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair AccI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 4 were converted to unique NotI sites using NotI linkers.

S-SPV-076

S-SPV-076 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli ß-galactosidase (lacZ) and the gene for pseudorabies virus (PRV) glycoprotein D (gD) and glycoprotein I (gI) were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the PRV gD and gI gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-076 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 829-55.16 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-076. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-076 was assayed for expression of PRV-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-PRV serum was shown to react specifically with S-SPV-076 plaques and not with S-SPV-003 negative control plaques. All S-SPV-076 observed plaques reacted with

the antiserum indicating that the virus was stably expressing the PRV gD protein.

S-SPV-076 is useful as a vaccine in swine against PRV infection. S-SPV-076 is also useful for expression of the PRV gD and gI proteins.

HOMOLOGY VECTOR 829-55.16. The plasmid 829-55.16 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and a pseudorabies virus (PRV) glycoprotein D (gD) and glycoprotein I (gI) gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß-galactosidase (lacZ) marker gene is under the control of a late promoter (LP1) and the PRV gD and gI genes are under the control of the late/early promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 500 base pair EcoRI to SalI restriction fragment derived from plasmid 538-46.16 (See WO95/03070). Fragment 3 is an approximately 1900 base pair SalI to BamHI restriction subfragment of PRV BamHI#7 genomic DNA fragment. Fragment 4 is an approximately 3010 base

pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 5 is an approximately 2149 base pair AccI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 5 were converted to unique NotI sites using NotI linkers.

S-SPV-079

S-SPV-079 is a swinepox virus that expresses two foreign genes. The gene for E. coli ß-galactosidase (lacZ) and the gene for pseudorabies virus (PRV) glycoprotein B (gB) were inserted into the unique HindIII restriction site (HindIII linkers inserted into a unique NdeI site in the SPV O1L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted). The lacZ gene is under the control of the synthetic late promoter (LP1), the PRV gB gene is under the control of the synthetic late/early promoter (LP2EP2). S-SPV-079 contains a PRV gB gene which codes for a protein of 913 amino acids, including 69 amino acids at the carboxy terminus which are missing in the PRV gB gene of S-SPV-015.

S-SPV-079 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 825-84.3 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 079. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods.

After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-079 was assayed for expression of PRV-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-PRV serum was shown to react specifically with S-SPV-079 plaques and not with S-SPV-003 negative control plaques. All S-SPV-079 observed plaques reacted with the antiserum indicating that the virus was stably expressing the PRV gB protein.

To confirm the expression of the PRV gB gene product, cells were infected with S-SPV-079 and samples of infected cell lysates were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal swine anti-PRV serum was used to detect expression of PRV specific proteins. The cell lysate from S-SPV-079 infected cells exhibited bands corresponding to 120 kd gB precursor and the 67 kd and 58 kd processed forms, which are the expected size of the PRV gB protein. PRV gB exists as a disulfide linked complex of these three forms.

S-SPV-079 is useful as a vaccine in swine against PRV infection. S-SPV-079 is also useful for expression of the PRV gB protein.

HOMOLOGY VECTOR 825-84.3. The plasmid 825-84.3 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and the pseudorabies virus (PRV) glycoprotein B (gB) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1532 base pair fragment of SPV DNA. Downstream of the foreign genes is

an approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the PRV gB gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1532 base pair BglII to NdeI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 2600 base pair EcoRI to SalI fragment derived from plasmid 727-54.60. Fragment 2 contains approximately 43 base pairs of synthetic DNA coding for PRV gB amino acids 1 to 16 and an approximately 2600 base pair SmaI to SalI fragment of PRV KpnI C genomic DNA. Fragment 3 is an approximately 210 base pair SalI to BamHI fragment generated by PCR which contains the coding sequence of the PRV gB gene. Fragment 3 contains the carboxy terminal 69 amino acids of PRV gB which are missing from S-SPV-015. Fragment 3 is an SalI to BamHI restriction fragment synthesized by polymerase chain reaction (PCR) using template DNA from PRV KpnI C genomic DNA. The primer (5′ ATGAAGGCCCTGTACCCCGTCACGA -3′; 11/95.3) **(SEQ ID NO 54)** synthesized across the SalI of the PRV gB gene and reproduced a SalI site internal to the gB gene. The primer (5′-CG<u>GGATCC</u>GGCTACAGGGCGTCGGGGTCCTC3′-3′; 11/95.4) **(SEQ ID NO 55)** was used for PCR and synthesized from the 3′ end of the PRV gB gene and introduced a BamHI site at the 3′ end of the PRV gB gene. The PCR product was digested

with SalI and BamHI to yield a fragment approximately 210 base pairs in length corresponding to the carboxy terminus of the PRV gB gene. Fragment 4 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 5 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The NdeI sites in fragments 1 and 5 were converted to unique HindIII sites using HindIII linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted which would span SPV fragments 1 and 5.

<u>S-SPV-090:</u>

S-SPV-090 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* ß-galactosidase (lacZ) and the gene for pseudorabies virus (PRV) glycoprotein I (gI) were inserted into the 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of nucleotides 1679 to 2452, SEQ ID NO: 189). The lacZ gene is under the control of the swinepox O1L promoter, and the PRV gI gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-090 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 837-58.14 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-090. This virus was assayed for ß-galactosidase expression, purity, and insert

stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-090 is useful as a vaccine in swine against PRV infection. S-SPV-090 is also useful for expression of the PRV gI protein.

HOMOLOGY VECTOR 837-58.14. The plasmid 837-58.14 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lac Z) marker gene and an pseudorabies virus (PRV) glycoprotein I (gI) gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 855 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1113 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the ß galactosidase (lacZ) marker gene is under the control of a swinepox virus 01L gene promoter and the PRV gI gene is under the control of the late/early promoter (LP2EP2). The LP2EP2 PRV gI gene cassette was inserted into a NotI site of homology vector 752-22.1. Homology vector 840-72.A1 was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2519 base pair HindIII to SphI restriction fragment of pSP65 (Promega). Fragment 1 is an approximately 855 base pair sub-fragment of the SPV HindIII restriction fragment M (23) synthesized by polymerase chain reaction using DNA primers 5' GAAGCATGCCCGTTCTTATCAATAGTTTAGTCGAAAATA-3' and 5'-

CATAAGATCTGGCATTGTGTTATTATACTAACAAAAATAAG-3′ to produce an 855 base pair fragment with SphI and BglII ends. Fragment 2 is a 3002 base pair *Bam*HI to *Pvu*II fragment derived from plasmid pJF751 (49) containing the *E. coli* lacZ gene. Fragment 3 is an approximately 1150 base pair BamHI fragment coding for the PRV gI gene derived by polymerase chain reaction (PCR) (Sambrook, et al., 1989) using the PRV BamHI#7 DNA fragment (pSY 138.-09.W) as template for the PCR reaction. To synthesize PRV gI, the primer (5′-CCGGATCCGGCGCGCGACGTGACCCGGCTC-3′; 11/95.1) **(SEQ ID NO 56)** synthesized from the 5′ end of the PRV gI gene and introduced a BamHI site at the 5′ end of the gene. The primer (5′-CCGGATCCGCGGACGGAGATAAAACGCCACCCAC -3′; 11/95.2) **(SEQ ID NO 57)** synthesized from the 3′ end of the PRV gI gene and introduced a BamHI site at the 3′ end of the gene. The PCR product was digested with BamHI to yield a fragment approximately 1150 base pairs in length corresponding to the PRV gI gene. Fragment 4 is an approximately 1113 base pair subfragment of the SPV HindIII fragment M synthesized by polymerase chain reaction using DNA primers 5′-CCGTAGTCGACAAAGATCGACTTATTAATATGTATGGGATT-3′ and 5′ GCCTGAAGCTTCTAGTACAGTATTTACGACTTTTGAAAT-3′ to produce an 1113 base pair fragment with SalI and HindIII ends.

Example 44: Homology V ctors Useful for Inserting Foreign DNA into the SPV HindIII K Genomic Region of a Recombinant Swinepox Virus

Plasmid 854-90.1 was constructed for insertion of foreign DNA into a recombinant swinepox virus. Plasmid 854-90.1 was constructed by changing the unique *Eco* RI site within the SPV *Hind* III K genomic fragment (Nucleotides: SEQ ID NO: 1) to a unique *Not* I restriction site through use of a DNA linker. The homology vector 854-90.1 contains an 1652 base pair region of SPV DNA upstream of the *Not* I insertion site and 5058 base pair region of SPV DNA downstream of the *Not* I insertion site. A homology vector containing foreign DNA inserted into plasmid 854-90.1 is useful when combined with swinepox virus DNA by HOMOLOGOUS RECOMBINATION for the construction of recombinant swinepox viruses.

Plasmid 855-37.5 was constructed for insertion of foreign DNA into a recombinant swinepox virus. Plasmid 855-37.5 was constructed by inserting an approximately 1875 base pair *Dra* I restriction fragment within swinepox virus HindIII K genome fragment from plasmid 854-90.1 containing the unique *Not* I insertion site, into plasmid PNEB193. The homology vector 855-37.5 contains an approximately 881 base pair region of SPV DNA upstream of the *Not* I insertion site and an approximately 994 base pair region of SPV DNA downstream of the *Not* I insertion site. The total size of homology vector 855-37.5 is approximately 3.9 kb making it ideal for the insertion of two or more foreign genes into the homology vector and by homologous recombination into a recombinant swinepox virus.

Plasmid 847-42.2C was constructed for insertion of foreign DNA into a recombinant swinepox virus. Plasmid 847-42.2C was constructed by inserting the *uidA* gene into the unique *Eco*RI site within the SPV *Hin*dIII K genomic fragment. The *uidA* gene is under the control of the synthetic pox promoter, EP2. Additional foreign DNA is inserted upstream of the *uidA* gene into unique restriction sites *Not*I, *Sfi*I and *Xho*I.

Plasmid 847-42.7B was constructed for insertion of foreign DNA into a recombinant swinepox virus. Plasmid 847-42.2C was constructed by inserting the uidA gene into the unique EcoRI site within the SPV HindIII K genomic fragment. The uidA gene is under the control of the synthetic early promoter, EP2. Additional foreign DNA is inserted downstream of the uidA gene into unique restriction sites *Not*I, *Sfi*I and *Xho*I.

S-SPV-120:

S-SPV-120 is a swinepox virus that expresses two foreign genes. The gene for E. coli ß-galactosidase (lacZ) was inserted a unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment. The gene for E. coli ß-glucuronidase (uidA) was inserted into a unique NotI site (NotI linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region (SEQ ID NO: 1) of the 6.7 kb SPV HindIII K fragment). The lacZ gene is under the control of the synthetic late promoter (LP1), the uidA gene is under the control of the synthetic early promoter (EP2).

S-SPV-120 was derived from S-SPV-003 (Kasza Strain). This was accomplished utilizing the homology vector 847-42.2C (see above) and virus S-SPV-003 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING

RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase or ß-glucuronidase (BLUOGAL AND CPRG ASSAYS and SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of red plaque purification was the recombinant virus designated S-SPV 120. This virus was assayed for ß-galactosidase and ß-glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign genes.

S-SPV-120 is useful for inserting additional foreign DNA sequences into recombinant swinepox virus using white plaque selection in the presence of BLUOGAL or X-GLUC to selection for foreign DNA insertion into the unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment and the unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment.

Some examples of recombinant swinepox viruses expressing foreign DNA in the unique AccI restriction site in the O1L ORF of the HindIII M fragment and the unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment are: Recombinant SPV expressing swine influenza virus hemagglutinin, neuraminidase, and nucleoprotein; Recombinant SPV expressing porcine reproductive and respiratory disease virus ORF 5 and ORF6; Recombinant SPV expressing porcine reproductive and respiratory disease virus ORF2, ORF3, ORF4, ORF 5 and ORF6; Recombinant SPV expressing feline immunodeficiency virus gag/protease and envelope; Recombinant SPV

expressing feline leukemia virus gag/protease and envelope; Recombinant SPV expressing feline immunodeficiency virus gag/protease and envelope and feline leukemia virus gag/protease and envelope; Recombinant SPV expressing infectious bovine rhinotracheitis glycoprotein B, glycoprotein D, and glycoprotein I.

Additional examples of foreign DNA insertion sites in recombinant swinepox virus are exemplified by, but not limited to, one or more of the following: the unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment; the unique NdeI restriction site in the O1L ORF of the SPV HindIII M fragment (See Example S-SPV-052); the unique BglII restriction site within the 2.0 kb BglII to HindIII subfragment of the SPV HindIII M fragment (See Example S-SPV-047); the unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment (See Example S-SPV-059); the unique XhoI restriction site within the SPV HindIII J fragment (See Example S-SPV-064); the unique BglII restriction site within the SPV HindIII N fragment (See Example S-SPV-062); the unique EcoRV restriction site within the SPV HindIII N fragment (See Example S-SPV-060); the unique SnaBI restriction site within the SPV HindIII N fragment (See Example S-SPV-061).

Example 45: Recombinant swinepox virus expressing swine influenza virus genes in the SPV HindIII M and SPV HindIII K insertion sites

A recombinant swinepox virus expresses four foreign genes. The gene for swine influenza virus (SIV) hemagluttinin (HA) (H1N1) and the gene for E. coli ß-galactosidase (lacZ) were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced

a unique AccI restriction site).The gene for swine influenza virus (SIV) neuraminidase (NA) and the gene for E. coli ß-glucuronidase (uidA) were inserted into a unique NotI site (NotI linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region (SEQ ID NO: 1) of the 6.7 kb SPV HindIII K fragment). The SIV HA (H1N1) gene is under the control of the synthetic late/early promoter (LP2EP2), the SIV NA gene is under the control of the synthetic late/early promoter (LP2EP2), the lacZ gene is under the control of the synthetic late promoter (LP1), and the uidA gene is under the control of the synthetic early promoter (EP2).

The recombinant swinepox virus expressing swine influenza virus genes in the SPV HindIII M and SPV HindIII K insertion sites is derived from S-SPV-065 (Kasza Strain). This is accomplished utilizing the homology vector (with the SIV NA and E. coli uidA genes inserted into a unique NotI site in plasmid 855-37.5 (see above)) and virus S-SPV-065 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase or ß-glucuronidase (BLUOGAL AND CPRG ASSAYS and SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of red plaque and blue plaque purification is the recombinant swinepox virus. This virus was assayed for ß-galactosidase and ß-glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign genes.

Recombinant swinepox virus expressing swine influenza virus genes in the SPV HindIII M and SPV HindIII K insertion sites is useful as a vaccine in swine against SIV infection and is also useful for expression of the SIV HA and NA proteins.

S-SPV-121:

S-SPV-121 is a swinepox virus that expresses at least one foreign gene. The gene for swine influenza virus (SIV) hemagluttinin (HA) (H1N1) was inserted into a a unique NotI site (NotI linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region (SEQ ID NO: 1) of the 6.7 kb SPV HindIII K fragment). The SIV HA gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-121 is derived from S-SPV-059 (Kasza Strain). This is accomplished utilizing the homology vector with the SIV HA gene into a unique NotI site in plasmid 855-37.5 (see above) and virus S-SPV-059 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ENZYMATIC MARKER GENES (X-GLUC ASSAY). The final result of white plaque purification is the recombinant virus designated S-SPV-121. This virus is assayed for the absence of ß-glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are white indicating that the virus is pure, stable, and expressing the foreign genes.

S-SPV-121 is useful as a vaccine in swine against SIV infection S-SPV-121 is also useful for expression of the SIV HA protein.

S-SPV-122:

S-SPV-122 is a swinepox virus that expresses two foreign genes. The gene for swine influenza virus (SIV) hemagluttinin (HA) (H1N1) and neuraminidase (NA) were inserted into a unique NotI site (NotI linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region (SEQ ID NO: 1) of the 6.7 kb SPV HindIII K fragment). The SIV HA gene is under the control of the synthetic late/early promoter (LP2EP2) and the SIV NA gene is under the control of the synthetic early late promoter (EP2LP2).

S-SPV-122 is derived from S-SPV-059 (Kasza Strain). This is accomplished utilizing the homology vector with the SIV HA and NA genes inserted into a unique NotI site in plasmid 855-37.5 (see above) and virus S-SPV-059 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES. The final result of white plaque purification is the recombinant virus designated S-SPV-122. This virus is assayed for the absence of ß-glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are white indicating that the virus is pure, stable, and expressing the foreign genes.

S-SPV-122 is useful as a vaccine in swine against SIV infection S-SPV-122 is also useful for expression of the SIV HA and NA proteins.

Example 46: Recombinant swinepox virus expressing porcine reproductive and respiratory syndrome virus gen s in the SPV HindIII M and SPV HindIII K insertion sites

A recombinant swinepox virus expresses four foreign genes. The gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF5 and the gene for E. coli ß-galactosidase (lacZ) were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF6 and the gene for E. coli ß-glucuronidase (uidA) were inserted into a unique NotI site (NotI linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region (SEQ ID NO: 1) of the 6.7 kb SPV HindIII K fragment). The PRRS ORF5 gene is under the control of the synthetic late/early promoter (LP2EP2), the PRRS ORF6 gene is under the control of the synthetic late/early promoter (LP2EP2), the lacZ gene is under the control of the synthetic late promoter (LP1), and the uidA gene is under the control of the synthetic early promoter (EP1),

The recombinant swinepox virus expressing porcine reproductive and respiratory syndrome virus virus genes in the SPV HindIII M and SPV HindIII K insertion sites is derived from S-SPV-095 (Kasza Strain). This is accomplished utilizing the homology vector (with the PRRS ORF6 and E. coli uidA genes inserted into a unique NotI site in plasmid 855-37.5 (see above)) and virus S-SPV-095 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase or ß-glucuronidase (BLUOGAL AND CPRG ASSAYS and SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of

red plaque and blue plaque purification is the recombinant swinepox virus. This virus was assayed for ß-galactosidase and ß-glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign genes.

A recombinant swinepox virus expresses four foreign genes. The gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF6 and the gene for E. coli B-galactosidase (lacZ) are inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a uniques AccI restriction site.) The gene for porcine reproductive and respiratory system virus (PRRS) ORF5 and the gene for E. coli B-glucuronidase (uidA) are inserted into a unique NotI site (NotI linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region (SEQ ID NO 1) of the 6.7 kb SPV HindIII k fragment). The PRRS ORF6 gene is under the control of the synthetic late/early promoter (LP2EP2), the PRRS ORF5 gene is under the control of the synthetic late/early promoter (LP2EP2), the lacZ gene is under the control of the synthetic late promoter (LP1), and the uidA gene is under the control of the synthetic early promoter (EP2).

The recombinant swinepox virus expressing porcine reproductive and respiratory syndrome virus virus genes in the SPV HindIII M and SPV HindIII K insertion sites is derived from S-SPV-084 (Kasza Strain). This is accomplished utilizing the homology vector (with the PRRS ORF5 and E. coli uidA genes inserted into a unique NotI site in plasmid 855-37.5 (see above) and virus S-SPV-084 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR

GENERATING RECOMBINANT SPV. The transfection stock in screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING B-galactosidase or B-glucuronidase (BLUOGAL AND CPRG ASSAYS and SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of red plaque and blue plaque purification is the recombinant swinepox virus. This virus is assayed for B-galactosidase and B-glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign genes.

The recombinant swinepox virus expressing porcine reproductive and respiratory syndrome virus genes in the SPV HindIII M and SPV HindIII K insertion sites is useful as a vaccine in swine against PRRS infection and is also useful for expression of the PRRS ORF5 and ORF6 protein.

<u>Example 47:</u> **Recombinant swinepox virus expressing bovine viral diarrhea virus type 1 and type 2 genes in the SPV HindIII M and SPV HindIII K insertion sites**

<u>S-SPV-132</u>

S-SPV-132 is a recombinant swinepox virus which expresses four foreign genes. The gene for bovine viral diarrhea virus type 1 (BVDV-1) glycoprotein 53 (gp53) and the gene for E. coli B-galactosidase (lacZ) are inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site.) The gene for bovine viral diarrhea virus type 2 (BVDV-2) glycoprotein 53 (gp53) and the gene for E. coli B-glucuronidase (uidA) are inserted into a unique

NotI site (NotI linkers are inserted into a unique EcoRI restriction site within an approximately 3.2 kb region (SEQ ID NO 1) OF THE 6.7 KB SPV HindIII K fragment). Teh BVDV-1 gp53 gene and the BVDV-2 gp53 gene are under the control of the synthetic late/early promoter (LP2EP2), the lacZ gene is under the control of the synthetic late promoter (LP1), and the uidA gene is under the control of the synthetic early promoter (EP2).

S-SPV-132 is derived from S-SPV-051 (Kasza Strain). This is accomplished utilizing the homology vector (with the BVDV-2 gp53 and E. coli uidA genes inserted into a unique NotI site in plasmid 855-37.5 (see above)) and virus S-SPV-051 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock is screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING B-galactosidase or B-glucuronidase (BLUOGAL AND CPRG ASSAYS and SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of red plaque and blue plaque purification is the recombinant swinepox virus. This virus is assayed for B-galactosidase and B-glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign genes.

S-SPV-132 is useful as a vaccine in swine against BVDV infection and is also useful for expression of the BVDV-1 gp53 and BVDV-2 gp53.

S-SPV-134:

S-SPV-134 is a swinepox virus that expresses four foreign genes. The gene for porcine reproductive and

respiratory syndrome virus (PRRS) ORF6 and the gene for E. coli ß-galactosidase (lacZ) were inserted into a unique Not I restriction site (NotI linkers inserted into a unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment). The gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF5 and the gene for E. coli ß-glucuronidase (uidA) were inserted into a unique NotI site (NotI linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment). The porcine reproductive and respiratory syndrome virus (PRRS) ORF6 gene is under the control of the synthetic late/early promoter (LP2EP2), the porcine reproductive and respiratory syndrome virus (PRRS) ORF5 gene is under the control of the synthetic late/early promoter (LP2EP2). The lacZ gene is under the control of the synthetic late promoter (LP1), the uidA gene is under the control of the synthetic early promoter (EP2).

S-SPV-134 was derived from S-SPV-084 (Kasza Strain). This was accomplished utilizing the homology vector 855-52.31 and virus S-SPV-084 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-glucuronidase (SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of blue plaque purification was the recombinant virus designated S-SPV 134 . This virus was assayed for ß-galactosidase and ß-glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign genes.

To confirm the expression of the porcine reproductive and respiratory syndrome virus (PRRS) ORF5 and ORF6 gene products, cells were infected with S-SPV-134 and samples of infected cell lysates were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal swine anti-PRRS (NVSL) serum was used to detect expression of PRRS specific proteins. The cell lysate from SPV-134 infected cells exhibited bands corresponding to 26 kd and 18 kd, which is the expected size of the SIV ORF5 and ORF6 protein. A 40 kd band, representing a heterodimer formed between ORF5 and ORF6 protein, was also seen under nonreducing conditions suggesting the formation of a disulfide-linked complex between ORF5 and ORF6 proteins. The assay described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

S-SPV-134 is a recombinant swinepox virus expressing the PRRS ORF5 and ORF6 proteins and is useful as a vaccine in swine against PRRS infection. S-SPV-134 is also useful for expression of the PRRS ORF5 and ORF6 proteins.

HOMOLOGY VECTOR 855-52.31. The homology vector 855-52.31 was used to insert foreign DNA into SPV. It incorporates an E. coli ß-glucuronidase (uidA) marker gene and the porcine reproductive and respiratory syndrome virus (PRRS) ORF5 gene flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the ß-glucuronidase (uidA) marker gene is under the control of a synthetic early pox promoter (EP2) and the porcine reproductive and respiratory syndrome virus (PRRS) ORF5 gene is

under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. Plasmid 855-52.31 was constructed using plasmid 847-42.7C. This plasmid was previously constructed by inserting the uidA gene into the unique EcoRI site within the SPV HindIII K genomic fragment. The uidA gene is under the control of the synthetic early promoter (EP2). The PRRS ORF5 gene was then inserted into a unique NotI site located upstream of the uidA gene resulting in plasmid 855-52.31. The transcriptional and translational orientation of the PRRS ORF5 gene is the same as the uidA gene.

The PRRS ORF5 gene is an approximately 603 base pair EcoRI to BamHI fragment synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using RNA from the PRRS NVSL reference strain. The upstream primer (5'-GAAGGATCCTAAGGACGACCCCATTGTTCCGCTG-3') synthesizes from the 5' end of the PRRS ORF5 gene and introduces an EcoRI site at the 5' end of the gene. The downstream primer was (5'-GCGGATCCTTGTTATGTGGCATATTTGACAAGGTTTAC-3') synthesizes from the 3' end of the PRRS ORF5 gene, introduces an BamHI site at the 3' end of the gene, and was used for reverse transcription and polymerase chain reaction. The PCR product was digested with EcoRI and BamHI to yield a fragment 603 base pairs in length corresponding to the PRRS ORF5 gene.

S-SPV-136 :

S-SPV-136 is a swinepox virus that expresses four foreign genes. The gene for porcine reproductive and

respiratory syndrome virus (PRRS) ORF6 and the gene for E. coli ß-galactosidase (lacZ) were inserted into a unique Not I restriction site (NotI linkers inserted into a unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment). The gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF5 and the gene for E. coli ß-glucuronidase (uidA) were inserted into a unique NotI site (NotI linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment). The porcine reproductive and respiratory syndrome virus (PRRS) ORF6 gene is under the control of the synthetic late/early promoter (LP2EP2), the porcine reproductive and respiratory syndrome virus (PRRS) ORF5 gene is under the control of the synthetic late/early promoter (LP2EP2). The lacZ gene is under the control of the synthetic late promoter (LP1), the uidA gene is under the control of the synthetic early promoter (EP2).

S-SPV-136 was derived from S-SPV-084 (Kasza Strain). This was accomplished utilizing the homology vector 855-52.43 and virus S-SPV-084 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-glucuronidase (SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of blue plaque purification was the recombinant virus designated S-SPV 136. This virus was assayed for ß-galactosidase and ß-glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign genes.

To confirm the expression of the porcine reproductive and respiratory syndrome virus (PRRS) ORF5 and ORF6 gene products, cells were infected with S-SPV-136 and samples of infected cell lysates were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal swine anti-PRRS (NVSL) serum was used to detect expression of PRRS specific proteins. The cell lysate from SPV-136 infected cells exhibited bands corresponding to 26 kd and 18 kd, which is the expected size of the SIV ORF5 and ORF6 protein. A 40 kd band, representing a heterodimer formed between ORF5 and ORF6 protein, was also seen under nonreducing conditions suggesting the formation of a disulfide-linked complex between ORF5 and ORF6 proteins. The assay described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

S-SPV-136 is a recombinant swinepox virus expressing the PRRS ORF5 and ORF6 proteins and is useful as a vaccine in swine against PRRS infection. S-SPV-136 is also useful for expression of the PRRS ORF5 and ORF6 proteins.

HOMOLOGY VECTOR 855-52.43. The homology vector 855-52.43 was used to insert foreign DNA into SPV. It incorporates an E. coli ß-glucuronidase (uidA) marker gene and the porcine reproductive and respiratory syndrome virus (PRRS) ORF5 gene flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the ß-glucuronidase (uidA) marker gene is under the control of a synthetic early pox promoter (EP2) and the porcine reproductive

and respiratory syndrome virus (PRRS) ORF5 gene is under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. Plasmid 855-52.43 was constructed using plasmid 847-42.7C. This plasmid was previously constructed by inserting the uidA gene into the unique EcoRI site within the SPV HindIII K genomic fragment. The uidA gene is under the control of the synthetic early promoter (EP2). The PRRS ORF5 gene was then inserted into a unique NotI site located upstream of the uidA gene resulting in plasmid 855-52.31. The transcriptional and translational orientation of the PRRS ORF5 gene is opposite of the uidA gene.

The PRRS ORF5 gene is an approximately 603 base pair EcoRI to BamHI fragment synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using RNA from the PRRS NVSL reference strain. The upstream primer (5'-GAAGGATCCTAAGGACGACCCCATTGTTCCGCTG-3') synthesizes from the 5' end of the PRRS ORF5 gene and introduces an EcoRI site at the 5' end of the gene. The downstream primer was (5'-GCGGATCCTTGTTATGTGGCATATTTGACAAGGTTTAC-3') synthesizes from the 3' end of the PRRS ORF5 gene, introduces an BamHI site at the 3' end of the gene, and was used for reverse transcription and polymerase chain reaction. The PCR product was digested with EcoRI and BamHI to yield a fragment 603 base pairs in length corresponding to the PRRS ORF5 gene.

S-SPV-157:

S-SPV-157 is a swinepox virus that expresses three foreign genes. The gene for swine influenza virus (SIV) neuraminidase (NA) (H1N1) and the gene for E. coli

ß-galactosidase (lacZ) were inserted into a unique Not I restriction site (Not I linkers inserted into a unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment). The gene for swine influenza virus (SIV) hemagluttinin (HA) (H1N1) was inserted into a unique Not I site (Not I linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment). The NA gene is under the control of the synthetic late/early promoter (LP2EP2), The HA gene is under the control of the synthetic late/early promoter (LP2EP2), and the lacZ gene is under the control of the synthetic late promoter (LP1).

S-SPV-157 was derived from S-SPV-121 (Kasza Strain). This was accomplished utilizing the homology vector 807-84.35 and virus S-SPV-121 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS and SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of red plaque purification was the recombinant virus designated S-SPV-157. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign genes.

To confirm the expression of the SIV HA and NA gene products, cells were infected with S-SPV-157 and samples of infected cell lysates and culture supernatents were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal goat

anti-HA or a polyclonal goat anti-NA serum was used to detect expression of SIV specific proteins. The cell lysate from SPV-157 infected cells exhibited bands corresponding to 64 kd and 52 kd, which is the expected size of the SIV HA and NA protein.

S-SPV-157 was assayed for expression of SIV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-SIV or polyclonal goat anti-HA serum was shown to react specifically with S-SPV-157 plaques and not with S-SPV-001 negative control plaques. All S-SPV-157 observed plaques reacted with both serums indicating that the virus was stably expressing the SIV foreign genes. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

S-SPV-157 is a recombinant swinepox virus expressing the SIV HA and NA proteins and is useful as a vaccine in swine against SIV infection. S-SPV-157 is also useful for expression of the SIV HA and NA proteins.

HOMOLOGY VECTOR 807-84.35. The homology vector 807-84.35 was used to insert foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and the swine influenza virus (SIV) neuraminidase (NA) gene flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the ß galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1) and the SIV NA gene is under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard

recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII fragment M (23). Fragment 2 is an approximately 1414 base pair EcoRI to BglII fragment of the SIV NA gene synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using RNA from the SIV H1N1 strain (NVSL). The upstream primer (5'-AATGAATTCAAATCAAAAAATAATAACCATTGGGTCAAT-3') synthesizes from the 5' end of the SIV NA gene and introduces an EcoRI site at the 5' end of the gene. The downstream primer was (5'-GGAAGATCTACTTGTCAATGGTGAATGGCAGATCAG-3') synthesizes from the 3' end of the SIV NA gene, introduces an BglII site at the 3' end of the gene, and was used for reverse transcription and polymerase chain reaction. The PCR product was digested with EcoRI and BglII to yield a fragment 1414 base pairs in length corresponding to the SIV NA gene. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair AccI to HindIII restriction sub-fragment of the SPV HindIII restriction fragment M (23). The AccI site in the SPV homology vector was converted to a unique NotI site using synthetic linkers.

<u>S-SPV-158:</u>

S-SPV-158 is a swinepox virus that expresses four foreign genes. The gene for swine influenza virus (SIV) nucleoprotein (NP) (H1N1) and the gene for E. coli ß-galactosidase (lacZ) were inserted into a unique Not I restriction site (Not I linkers inserted into a

unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment). The genes for swine influenza virus (SIV) hemagluttinin (HA) (H1N1) and neuraminidase (NA) (H1N1) were inserted into a unique Not I site (Not I linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment). The NA gene is under the control of the synthetic late/early promoter (LP2EP2), the HA gene is under the control of the synthetic late/early promoter (LP2EP2), the NP gene is under the control of the synthetic late/early promoter (LP2EP2), and the lacZ gene is under the control of the synthetic late promoter (LP1).

S-SPV-158 was derived from S-SPV-122 (Kasza Strain). This was accomplished utilizing the homology vector 807-41.03 and virus S-SPV-122 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS and SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of red plaque purification was the recombinant virus designated S-SPV-158. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign genes.

To confirm the expression of the SIV HA, NA, and NP gene products, cells were infected with S-SPV-158 and samples of infected cell lysates and culture supernatients were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal goat

anti-HA, a polyclonal goat anti-NP, or a polyclonal goat anti-NA serum was used to detect expression of SIV specific proteins. The cell lysate from SPV-158 infected cells exhibited bands corresponding to 64 kd, 52 kd, and 56 kd, which is the expected size of the SIV HA, NA, and NP proteins, respectively.

S-SPV-158 was assayed for expression of SIV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. A polyclonal swine anti-SIVserum, a polyclonal goat anti-HA serum, or a polyclonal goat anti-NP serum was shown to react specifically with S-SPV-158 plaques and not with S-SPV-001 negative control plaques. All S-SPV-158 observed plaques reacted with all three serological reagents indicating that the virus was stably expressing the SIV foreign genes. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

S-SPV-158 is a recombinant swinepox virus expressing the SIV HA, NA, and NP proteins and is useful as a vaccine in swine against SIV infection. S-SPV-158 is also useful for expression of the SIV HA, NA, and NP proteins.

HOMOLOGY VECTOR 807-41.03. The homology vector 807-41.03 was used to insert foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and the swine influenza virus (SIV) nucleoprotein (NP) gene flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the ß galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter

(LP1) and the SIV NP gene is under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII fragment M (23). Fragment 2 is an approximately 1501 base pair EcoRI to EcoRI fragment of the SIV NP gene synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using RNA from the SIV H1N1 strain (NVSL). The upstream primer (5'-CATGAATTCTCAAGGCACCAAACGATCATATGAAC-3') synthesizes from the 5' end of the SIV NP gene and introduces an EcoRI site at the 5' end of the gene. The downstream primer was (5'-ATTTGAATTCAATTGTCATACTCCTCTGCATTGTCT-3') synthesizes from the 3' end of the SIV NP gene, introduces an EcoRI site at the 3' end of the gene, and was used for reverse transcription and polymerase chain reaction. The PCR product was digested with EcoRI to yield a fragment 1501 base pairs in length corresponding to the SIV NP gene. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair AccI to HindIII restriction sub-fragment of the SPV HindIII restriction fragment M (23). The AccI site in the SPV homology vector was converted to a unique NotI site using synthetic linkers.

S-SPV-217:

S-SPV-217 is a swinepox virus that expresses four foreign genes. The gene for porcine reproductive and

respiratory syndrome virus (PRRS) ORF5 and the gene for E. coli ß-galactosidase (lacZ) are inserted into a unique Not I restriction site (NotI linkers inserted into a unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment). The gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF6 and the gene for E. coli ß-glucuronidase (uidA) are inserted into a unique NotI site (NotI linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment). The porcine reproductive and respiratory syndrome virus (PRRS) ORF6 gene is under the control of the synthetic early promoter (EP1), the porcine reproductive and respiratory syndrome virus (PRRS) ORF5 gene is under the control of the synthetic early promoter (EP2). The lacZ gene is under the control of the synthetic late promoter (LP1), the uidA gene is under the control of the synthetic early promoter (EP2).

S-SPV-217 is derived from S-SPV-174 (Kasza Strain). This was accomplished utilizing a homology vector and virus S-SPV-174 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock is screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-glucuronidase (SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of blue plaque purification is the recombinant virus designated S-SP-217. This virus is assayed for ß-galactosidase and ß-glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods.

S-SPV-217 is a recombinant swinepox virus expressing the PRRS ORF5 and ORF6 proteins and is useful as a vaccine in swine against PRRS infection. S-SPV-134 is

also useful for expression of the PRRS ORF5 and ORF6 proteins.

S-SPV-218:

S-SPV-218 is a swinepox virus that expresses four foreign genes. The gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF5 and the gene for E. coli ß-galactosidase (lacZ) are inserted into a unique Not I restriction site (NotI linkers inserted into a unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment). The gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF6 and the gene for E. coli ß-glucuronidase (uidA) are inserted into a unique NotI site (NotI linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment). The porcine reproductive and respiratory syndrome virus (PRRS) ORF6 gene is under the control of the synthetic late promoter (LP1), the porcine reproductive and respiratory syndrome virus (PRRS) ORF5 gene is under the control of the synthetic early promoter (EP2). The lacZ gene is under the control of the synthetic late promoter (LP1), the uidA gene is under the control of the synthetic early promoter (EP2).

S-SPV-218 is derived from S-SPV-174 (Kasza Strain). This was accomplished utilizing a homology vector and virus S-SPV-174 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock is screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-glucuronidase (SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of blue plaque purification is the recombinant virus designated S-SP-218. This virus is assayed for ß-galactosidase and ß-glucuronidase expression, purity, and insert stability by multiple

passages monitored by the blue plaque assay as described in Materials and Methods.

S-SPV-218 is a recombinant swinepox virus expressing the PRRS ORF5 and ORF6 proteins and is useful as a vaccine in swine against PRRS infection. S-SPV-134 is also useful for expression of the PRRS ORF5 and ORF6 proteins.

S-SPV-195:

S-SPV-195 is a swinepox virus that expresses one foreign gene. The full length gene encoding the surface and transmembrane protein subunits for FeLV (SU+TM) and the gene for E. coli ß-galactosidase (lacZ) were inserted into a unique Not I restriction site (Not I linkers inserted into a unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment). The FeLV envelope gene is under the control of the synthetic early promoter (EP1) and the lacZ gene is under the control of the synthetic late promoter (LP1).

S-SPV-195 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 911-4.A1 and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase. (BLUOGAL AND CPRG ASSAYS and SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of red plaque purification was the recombinant virus designated S-SPV 195. This virus was assayed for ß-galactosidase expression and is currently under going multiple passages to determine purity and stability.

S-SPV-195 was assayed for expression of FeLV envelope, gp70, using the BLACK PLAQUE SCREEN FOR FOREIGN GENE

EXPRESSION IN RECOMBINANT SPV. Monoclonal mouse anti-FeLV gp70 was shown to react specifically with S-SPV-195 plaques and not with S-SPV-001 negative control plaques. All S-SPV-195 plaques observed reacted with the monoclonal antibody indicating that the virus was stably expressing the FeLV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

S-SPV-195 is a recombinant swinepox virus expressing the FeLV envelope proteins and is useful as a vaccine in cats against feline leukemia infection. S-SPV-195 is also useful for expression of the FeLV envelope protein.

HOMOLOGY VECTOR 911-4.A1. The homology vector 911-4.A1 was used to insert foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and the full-length FeLV envelope gene flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the ß galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1) and the FeLV envelope gene is under the control of a synthetic early pox promoter (EP1). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV

HindIII fragment M (23). Fragment 2 is an approximately 1929 base pair EcoRI to BamHI fragment of the FeLV envelope gene synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using FeLV cDNA from the p61E, subtype A FeLV/FAIDs strain. The upstream primer (5'-CGTCGGATCCGGACAGCCCCAGCTTAGACGATC-3') synthesizes from the 5' end of the FeLV envelope gene and introduces an EcoRI site at the 5' end of the gene. The downstream primer (5'-CGTCGGATCCGGGACTAAATGGAATCATACA-3') synthesizes from the 3' end of the FeLV envelope gene, and introduces a BamHI site at the 3' end of the gene. These primers were used for reverse transcription and polymerase chain reaction by CLONING WITH THE POLYMERASE CHAIN REACTION. The PCR product was digested with EcoRI and BamHI to yield a fragment of approximately 1929 base pairs in length corresponding to the FeLV envelope gene.

Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair AccI to HindIII restriction sub-fragment of the SPV HindIII restriction fragment M (23). The AccI site in the SPV homology vector was converted to a unique Not I site using synthetic linkers.

S-SPV-205:

S-SPV-205 is a swinepox virus that expresses four foreign genes. The gene for feline leukemia virus (FeLV) gag/protease the gene for E. coli ß-galactosidase (lacZ) were inserted into a unique Not I restriction site (Not I linkers inserted into a unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment). The gene for FeLV envelope and ß-glucuronidase (uidA) were inserted into a unique Not

I site (Not I linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment). The FeLV gag/protease gene is under the control of the synthetic late/early promoter (LP2EP2), and the FeLV envelope gene is under the control of a synthetic early promoter (EP1). The lacZ gene is under the control of the synthetic late promoter, LP1 and the uidA gene is under the control of the synthetic early pox promoter, EP2.

S-SPV-205 was derived from S-SPV-089 (Kasza Strain). This was accomplished by utilizing the homology vector 905-31.A1 and virus S-SPV-089 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-glucoronidase (X-GLUC and SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). Recombinant plaques expressing the marker gene were shown to be positive for ß-glucuronidase by blue/green plaque detection and were designated as SPV 205.

This virus was assayed for ß-glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue/green plaque assay as described in Materials and Methods. After the initial three rounds of passage, all plaques observed are blue/green, indicating that the virus is pure. Analysis of expression of the FeLV gag and envelope genes and stability analysis are in progress.

HOMOLOGY VECTOR 905-31.A1. The homology vector 905-31.A1 was used to insert foreign DNA into SPV089. It incorporates an E. coli ß-glucuronidase (uidA) marker gene and the FeLV envelope gene flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING

RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the ß-glucuronidase (uidA) marker gene is under the control of a synthetic early pox promoter (EP2) and the FeLV envelope gene is under the control of a separate and unique synthetic early pox promoter (EP1). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. Plasmid 905-31.A1 was constructed using plasmid 847-42.2C. This plasmid was previously constructed by inserting the uidA gene into the unique EcoRI site within the SPV HindIII K genomic fragment. The FeLV envelope gene was then inserted into a unique Not I site located upstream of the uidA gene resulting in plasmid 905-31.A1.
The FeLV envelope gene is an approximately 1929 base pair EcoRI to BamHI fragment synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using FeLV cDNA from the p61E, subtype A FeLV/FAIDs strain. The upstream primer (5'-CGTCGGATCCGGACAGCCCCAGCTTAGACGATC-3') synthesizes from the 5′ end of the FeLV envelope gene and introduces an EcoRI site at the 5′ end of the gene. The downstream primer (5′-CGTCGGATCCGGGGACTAAATGGAATCATACA-3′) synthesizes from the 3′ end of the FeLV envelope gene, and introduces a BamHI site at the 3′ end of the gene. These primers were used for reverse transcription and polymerase chain reaction. The PCR product was digested with EcoRI and BamHI to yield a fragment of approximately 1929 base pairs in length corresponding to the FeLV envelope gene.

S-SPV-197:

S-SPV-197 is a swinepox virus that expresses one foreign gene. The full-length envelope gene from feline

leukemia virus, subtype A (FeLV) and the gene encoding E. coli ß-glucuronidase (uidA) were inserted into a unique Not I restriction site (Not I linkers inserted into a unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment). The gene for FeLV envelope was inserted into a unique Not I site (Not I linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment). The envelope gene is under the control of the synthetic early promoter (EP2) and the uidA gene is under the control of a separate and unique synthetic early promoter (EP2).

S-SPV-197 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 905-31.A1 and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-glucoronidase (X-GLUC and SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of blue/green purification was the recombinant virus designated S-SPV-197.

This virus was assayed for ß-glucoronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue/green indicating that the virus is pure, stable, and expressing the foreign genes.

To confirm the expression of the FeLV envelope foreign gene product, cells were infected with S-SPV-197 and samples of infected cell lysates were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A

mouse monoclonal anti-FelVgp70 antibody was used to detect expression of FeLV gp70 specific protein. The cell lysate from SPV-195 infected cells exhibited bands corresponding to 85kda and 70kda, which are the expected sizes for the unprocessed FeLV envelope protein and the processed gp 70 envelope surface protein. The 70kda protein product was predominant in the cell lysates.

S-SPV-197 was assayed for expression of FeLV envelope, gp70, using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Monoclonal mouse anti-FeLV gp70 was shown to react specifically with S-SPV-197 plaques and not with S-SPV-001 negative control plaques. All S-SPV-197 plaques observed reacted with the monoclonal antibody indicating that the virus was stably expressing the FeLV envelope gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

S-SPV-197 is a recombinant swinepox virus expressing the FeLV envelope proteins and is useful as a vaccine in cats against feline leukemia infection. S-SPV-197 is also useful for expression of the FeLV envelope protein.

HOMOLOGY VECTOR 905-31.A1: The homology vector 905-31.A1 was used to insert foreign DNA into SPV. It incorporates an E. coli ß-glucuronidase (uidA) marker gene and the FeLV envelope gene flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes results. Note that the ß-glucuronidase (uidA) marker gene is under the control of a synthetic

early pox promoter (EP2) and the FeLV envelope virus gene is under the control of a separate and unique synthetic early pox promoter (EP1). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. Plasmid 905-31.A1 was constructed using plasmid 847-42.2C. This plasmid was previously constructed by inserting the uidA gene into the unique EcoRI site within the SPV HindIII K genomic fragment. The FeLV envelope gene was then inserted into a unique Not I site located upstream of the uidA gene resulting in plasmid 905-31.A1.

The FeLV envelope gene is an approximately 1929 base pair EcoRI to BamHI fragment synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using FeLV cDNA from the p61E, subtype A FeLV/FAIDs strain. The upstream primer (5'-CGTCGGATCCGGACAGCCCCAGCTTAGACGATC-3') synthesizes from the 5' end of the FeLV envelope gene and introduces an EcoRI site at the 5' end of the gene. The downstream primer (5'-CGTCGGATCCGGGGACTAAATGGAATCATACA-3') synthesizes from the 3' end of the FeLV envelope gene, and introduces a BamHI site at the 3' end of the gene. These primers were used for reverse transcription and polymerase chain reaction. The PCR product was digested with EcoRI and BamHI to yield a fragment of approximately 1929 base pairs in length corresponding to the FeLV envelope gene.

<u>S-SPV-198:</u>

S-SPV-198 is a swinepox virus that expresses two foreign genes. The full-length envelope gene from feline leukemia virus, subtype A (FeLV) and the gene

encoding E. coli ß-glucuronidase (uidA) were inserted into a unique Not I restriction site (Not I linkers inserted into a unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment). The gene for FeLV envelope was inserted into a unique Not I site (Not I linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment). The ß-glucuronidase gene is under the control of the synthetic early pox promoter, EP2. The FeLV envelope gene is under the control of the early/late synthetic pox promoter, EP1. Note that the two promoter/gene cassettes are orientated in opposing directions, to avoid possible homologous recombination between identical promoter elements (EP2).

S-SPV-198 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 860-2.A5 and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-glucoronidase (X-GLUC and SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). Recombinant plaques expressing the marker gene were shown to be positive for ß-glucuronidase by blue/green plaque detection and are designated as SPV 198.

S-SPV-198 is in the process of purification, analysis of foreign gene expression and stability using black plaque and western blot assays, as described in Materials and Methods. The assays described here are carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

S-SPV-198 is a recombinant swinepox virus expressing the FeLV envelope protein and is useful as a vaccine in

cats against feline leukemia infection. S-SPV-198 is also useful for expression of the FeLV envelope protein.

HOMOLOGY VECTOR 860-2.A5. The homology vector 860-2.A5 was used to insert foreign DNA into SPV. It incorporates an E. coli ß-glucuronidase (uidA) marker gene and the FeLV envelope gene flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes results. Note that the ß-glucuronidase (uidA) marker gene is under the early synthetic pox promoter (EP2) and the FeLV envelope gene is under the control of a synthetic early/late pox promoter (LP2/EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. Plasmid 860-2.A5 was constructed using plasmid 847-42.2C. This plasmid was previously constructed by inserting the uidA gene into the unique EcoRI site within the SPV HindIII K genomic fragment. The FeLV envelope gene was then inserted into a unique NotI site located upstream of the uidA gene resulting in plasmid 860-2.A5.

The FeLV envelope gene is an approximately 1929 base pair EcoRI to BamHI fragment synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using FeLV cDNA from the p61E, subtype A, FeLV/FAIDs strain (received from the NIAIDS repository, cat.# 109). The upstream primer (5'-CGTCGGATCCGGACAGCCCCAGCTTAGACGATC-3') synthesizes from the 5′ end of the FeLV envelope gene and introduces an EcoRI site at the 5′ end of the gene. The downstream primer

introduces an EcoRI site at the 5' end of the gene. The downstream primer (5'-CGTCGGATCCGGGGACTAAATGGAATCATACA-3') synthesizes from the 3' end of the FeLV envelope gene, and introduces a BamHI site at the 3' end of the gene. These primers were used for reverse transcription and polymerase chain reaction. The PCR product was digested with EcoRI and BamHI to yield a fragment of approximately 1929 base pairs in length corresponding to the FeLV envelope gene.

S-SPV-206:

S-SPV-206 is a swinepox virus that expresses four foreign genes. The gene for FIV envelope and the gene for E. coli ß-galactosidase (lacZ) were inserted into a unique Not I restriction site (Not I linkers inserted into a unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment). The gene for FIV gag/protease and the gene for E.coli ß-glucuronidase(uidA) was inserted into a unique Not I site (Not I linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment). The FIV gag/protease and envelope genes are under the control of separate, and identical synthetic late/early promoters (LP2EP2).The lacZ gene is under the control of the synthetic late promoter (LP1) and the uidA gene is under the control of the synthetic early promoter (EP2).

S-SPV-206 was derived from S-SPV-048. This was accomplished utilizing the homology vector 913-11.4 and virus S-SPV-048 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-glucuronidase (X-GLUC ASSAYS and SCREEN FOR RECOMBINANT HERPESVIRUS

EXPRESSING ENZYMATIC MARKER GENES). The final result of blue/green purification was the recombinant virus designated S-SPV-206. This virus was assayed for ß-glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue/green plaque assay as described in Materials and Methods. After the initial three rounds of passage, all plaques observed were blue/green indicating that the virus is pure, stable, and expressing the foreign genes.

To confirm the expression of the FIV gag/protease and envelope gene products, cells were infected with S-SPV-206 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal cat anti-FIV PPR serum or a monoclonal mouse anti-FIVgag(P24) antibody was used to detect expression of FIV envelope and gag specific proteins. The cell lysate from SPV-200 infected cells exhibited bands corresponding expected size bands of the FIV gag/protease and envelope proteins.

S-SPV-200 was assayed for expression of FIV gag/protease specific antigen using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Monoclonal mouse anti-FIV gag (P24) antibody was shown to react specifically with S-SPV-206 plaques and not with S-SPV-001 negative control plaques. All S-SPV-206 observed plaques reacted with this antibody indicating that the virus was stably expressing the FIV gag/protease foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

S-SPV-206 is a recombinant swinepox virus expressing the FIV gag/protease and envelope proteins and is useful as a vaccine in cats against FIV infection. S-SPV-206 is also useful for expression of the FIV gag/protease and envelope genes.

HOMOLOGY VECTOR 913-11.4. The homology vector 913-11.4 was used to insert foreign DNA into SPV 048. It incorporates an E. coli ß-glucuronidase (uidA) marker gene and FIV gag/protease gene flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the uidA marker gene is under the control of a synthetic early pox promoter EP2 and the FIVgag/protease is under the control of a synthetic late/early pox promoter (LP2EP2). These two promoter/gene cassettes were oriented in opposing directions with the promoters adjacent, but in opposite orientations to avoid homologous recombination between identical EP2 promoter elements. The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. Plasmid 913-11.4 was constructed using plasmid 847-42.2C. This plasmid was previously constructed by inserting the uidA gene into the unique EcoRI site within the SPV HindIII K genomic fragment. The uidA gene is under the control of the synthetic early promoter (EP2). The FIVgag/protease gene was then inserted into a unique NotI site located upstream of the uidA gene resulting in plasmid 913-11.4.

The FIV gag/protease gene was synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using cDNA from the FIV PPR strain. The

upstream primer (5'-GCGTGAATTCGGGGAATGGACAGGGGCGAGAT-3') synthesizes from the 5' end of the FIV gag/protease gene and introduces an EcoRI site at the 5' end of the gene. The downstream primer was (5'-GAGCCAGATCTGCTCTTTTTACTTTCCC -3') synthesizes from the 3' end of the FIV gag/protease gene, introduces an BglII site at the 3' end of the gene, and was used for reverse transcription and polymerase chain reaction. The PCR product was digested with EcoRI and BglII to yield a fragment approximately 1839 base pairs in length corresponding to the FIV gag/protease gene.

__S-SPV-200:__

S-SPV-200 is a swinepox virus that expresses three foreign genes. The genes for feline immunodeficiency virus (FIV) gag/protease, and full length envelope and the gene for E. coli ß-galactosidase (lacZ) were inserted into a unique Not I restriction site (Not I linkers inserted into a unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment). The FIVgag/protease and envelope genes are under the control of separate, but identical synthetic late/early promoter (LP2EP2. The lacZ gene is under the control of the synthetic late promoter (LP1).

S-SPV-200 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 904-63.B7 and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS and SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of red plaque purification was the recombinant virus designated S-SPV-157. This virus was assayed for ß-galactosidase expression by the blue plaque assay as

described in Materials and Methods. Analysis of purity, and insert stability by multiple passages is in progress and will be monitored by the blue plaque assay as described in Materials and Methods.

S-SPV-200 is a recombinant swinepox virus expressing the FIVgag/protease and FIV envelope proteins and is useful as a vaccine in swine against SIV infection. S-SPV-200 is also useful for expression of the FIV gag/protease and envelope proteins.

HOMOLOGY VECTOR 904-63.B7. The homology vector 904-63.B7 was used to insert foreign DNA into SPV 001. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and the feline immunodeficiency virus (FIV) gag/protease and envelope genes flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the ß galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1) and the FIV gag/protease and envelope genes are under the control of separate, but identical synthetic late/early pox promoters (LP2EP2). The FIVgag/protease and FIV envelope promoter/gene cassettes are oriented in opposing directions such that transcription of the gag/protease and envelope genes runs toward each other to avoid the possibility of homologous recombination between identical promoters. The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV

HindIII fragment M (23). Fragment 2 is an approximately 2580 base pair EcoRI to BglII fragment of the FIV envelope gene synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using cDNA from the FIV PPR strain. The upstream primer (5'-GCCCGGATCCTATGGCAGAAGGGTTTGCAGC-3') synthesizes from the 5' end of the FIV envelope gene and introduces a BamHI site at the 5' end of the gene. The downstream primer was (5'-CCGTGGATCCGGCACTCCATCATTCCTCCTC -3') synthesizes from the 3' end of the FIV envelope gene, introduces an BamHI site at the 3' end of the gene, and was used for reverse transcription and polymerase chain reaction. The PCR product was digested with BamHI to yield a fragment 2580 base pairs in length corresponding to the FIV envelope gene. Fragment 3 is an approximately 1839 base pair EcoRI to BglII fragment of the FIV gag/protease gene synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using cDNA from the FIV PPR strain. The upstream primer (5'-GCGTGAATTCGGGGAATGGACAGGGGCGAGAT-3') synthesizes from the 5' end of the FIV gag/protease gene and introduces an EcoRI site at the 5' end of the gene. The downstream primer was (5'-GAGCCAGATCTGCTCTTTTTACTTTCCC -3') synthesizes from the 3' end of the FIV gag/protease gene, introduces an BglII site at the 3' end of the gene, and was used for reverse transcription and polymerase chain reaction. The PCR product was digested with EcoRI and BglII to yield a fragment approximately 1839 base pairs in length corresponding to the FIV gag/protease gene. Fragment 4 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 5 is an approximately 2149 base pair AccI to HindIII restriction sub-fragment of the SPV HindIII restriction fragment M (23). The AccI site in the SPV homology

vector was converted to a unique NotI site using synthetic linkers.

S-SPV-207:

S-SPV-207 is a swinepox virus that expresses four foreign genes. The gene for FIV gag/protease and the gene for E. coli ß-galactosidase (lacZ) were inserted into a unique Not I restriction site (Not I linkers inserted into a unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment). The gene for FIV envelope and the gene for E.coli ß-glucuronidase(uidA) was inserted into a unique Not I site (Not I linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment). The FIV gag/protease gene is under the control of the late/early promoters (LP2EP2).The lacZ gene is under the control of the constitutive SPV promoter, O1L. The FIV envelope gene is under the control of the synthetic early pox promoter (EP1) and the uidA gene is under the control of the synthetic early promoter (EP2).

S-SPV-207 was derived from S-SPV-046. This was accomplished utilizing the homology vector 911-96.A2 and virus S-SPV-046 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-glucuronidase (X-GLUC ASSAYS and SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of blue/green purification was the recombinant virus designated S-SPV-207. Initial virus purifications were assayed for ß-glucuronidase expression by the blue/green plaque assay as described in Materials and Methods. Final purification and expression analysis of foreign genes is in progress.

S-SPV-207 is a recombinant swinepox virus expressing the FIV gag/protease and envelope proteins and is useful as a vaccine in cats against FIV infection. S-SPV-207 is also useful for expression of the FIV gag/protease and envelope genes.

HOMOLOGY VECTOR 911-96.A2. The homology vector 911-96.A2 was used to insert foreign DNA into SPV 046. It incorporates an E. coli ß-glucuronidase (uidA) marker gene and FIV envelope gene flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the uidA marker gene is under the control of a synthetic early pox promoter (EP2) and the FIV envelope is under the control of a synthetic early pox promoter (EP1). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. Plasmid 911-96.A2 was constructed using plasmid 847-42.2C. This plasmid was previously constructed by inserting the uidA gene into the unique EcoRI site within the SPV HindIII K genomic fragment. The uidA gene is under the control of the synthetic early promoter (EP2). The FIV envelope gene was then inserted into a unique NotI site located upstream of the uidA gene resulting in plasmid 911-96.A2.

The FIV envelope gene was synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using cDNA from the FIV PPR strain. The upstream primer (5'-GCCCGGATCCTATGGCAGAAGGGTTTGCAGC -3') synthesizes from the 5' end of the FIV envelope gene and introduces a BamHI site at the 5' end of the gene. The downstream primer was

was used for reverse transcription and polymerase chain reaction. The PCR product was digested with BamHI to yield a fragment approximately 2580 base pairs in length corresponding to the FIV gag/protease gene.

S-SPV-142:

S-SPV-142 is a swinepox virus that expresses three foreign genes. The genes for bovine viral diarrhea virus type 1 (BVDV1) E2 glycoprotein and the gene for E. coli ß-galactosidase (lacZ) were inserted into a unique Not I restriction site (Not I linkers inserted into a unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment). The genes for bovine viral diarrhea virus type 1(BVDV1) E^{rns} glycoprotein, bovine viral diarrhea virus type 2(BVDV2) E2 glycoprotein and the E. coli ß-glucuronidase (uidA) marker gene were inserted into unique Bam HI, Not I and Pst I sites respectively (sites originating from a synthetic polylinker linker inserted into a unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment). The E2 genes are under the control of the synthetic late/early promoter (LP2EP2), The E^{rns} gene and the lacZ gene are under the control of the synthetic late promoter (LP1), and the uidA gene is under the control of the synthetic early promoter (EP2).

S-SPV-142 was derived from S-SPV-051 (Kasza Strain). This was accomplished utilizing the homology vector 874-4.6A and virus S-SPV-051 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The BVDV type 1 E2 gene and the lacZ gene in the Hind III M site were already present in virus S-SPV-051. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-glucuronidase (X-Gluc ASSAY and SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of green

plaque purification was the recombinant virus designated S-SPV-142. This virus was assayed for ß-glucuronidase expression, purity, and insert stability by multiple passages monitored by the green plaque assay as described in Materials and Methods. After the initial four rounds of purification, all plaques observed are green indicating that the virus is pure, stable, and expressing the foreign genes.

To confirm the expression of the BVDV type 2 E2 and type 1 E^{rns} gene products, cells were infected with S-SPV-142 and samples of infected cell lysates and culture supernatents were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A monoclonal mouse anti-E2 (type 2) or a monoclonal mouse anti-E^{rns} (type 1) serum was used to detect expression of BVDV-specific proteins. The cell lysate from S-SPV-142 infected cells exhibited bands corresponding to 53 kd and 42 kd, which is the expected size of the E2 glycoprotein (53 kd) but slightly smaller than the expected size of the E^{rns} glycoprotein (48 kd).

S-SPV-142 was assayed for expression of BVDV-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Monoclonal mouse anti-E2 sera were shown to react specifically with S-SPV-142 plaques and not with S-SPV-001 negative control plaques. All S-SPV-142 observed plaques reacted with both a type 1 E2 and a type 2 E2 serum indicating that the virus was stably expressing the BVDV E2 foreign genes. No reagent is currently available that reacts specifically with the E^{rns} glycoprotein in a black plaque assay. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

S-SPV-142 is a recombinant swinepox virus expressing the types 1&2 BVDV E2 glycoproteins and the type 1 E^{rns} glycoprotein and is useful as a vaccine in cattle against BVDV infection.

HOMOLOGY VECTOR 874-4.6A. The homology vector 874-4.6A was used to insert foreign DNA into SPV. It incorporates an E. coli ß-glucuronidase (uidA) marker gene and the bovine viral diarrhea virus (BVDV) type 1 E^{rns} gene and the BVDV type 2 E2 gene flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the ß-glucuronidase (uidA) marker gene is under the control of a synthetic early pox promoter (EP2), the E^{rns} gene is under control of the late synthetic pox promoter (LP1) and the BVDV E2 gene is under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. Plasmid 874-4.6A was constructed using plasmid 847-90.1A. This plasmid was previously constructed by inserting the BVDV type 2 E2 gene under control of the synthetic late/early promoter (LP2EP2) into a unique Not I site flanked by the EP2-uidA cassette downstream of the Not I site. The Not I site originated from a synthetic polylinker inserted into the unique Eco RI site of the SPV HindIII K genomic fragment. The EP2-uidA cassette had previously been inserted into a unique Pst I site within the same polylinker. The BVDV E^{rns} gene was inserted into the blunt-ended (filled in with a Klenow reaction) Bam HI site at the 3' terminus of the E2 gene resulting in plasmid 874-4.6A. The transcriptional and

translational orientation of the E^{rns} gene was the reverse of the E2 and uidA genes.

The BVDV E^{rns} gene is an approximately 744 base pair (63 bp signal sequence + 681 bp coding sequence) Eco RI to Bam HI fragment synthesized by reverse transcription (RT) and polymerase chain reaction using RNA from the BVDV1-Singer strain. The upstream primer (5'-CCATGAATTCGCTGGAAAAAGCATTGCTGGCATGGGC-3') synthesizes from the 5' end of the BVDV E^{rns} gene signal sequence and introduces an Eco RI site at the 5' end of the gene. The downstream primer (5'-TTCGGATCCTTACGCGTATGCTCCAAACCACGT-3') synthesizes from the 3' end of the E^{rns} gene and introduces a Bam HI site at the 3' end of the gene. The PCR product was digested with Eco RI and Bam HI to yield a fragment 744 base pairs in length corresponding to the BVDV1 E^{rns} gene.

S-SPV-187:

S-SPV-187 is a swinepox virus that expresses 2 foreign genes. The gene for Newcastle disease virus (NDV) F and the gene for E. coli ß-glucuronidase (uidA) were inserted into a unique Not1 site (Not1 linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment). The NDV F gene is under the control of the synthetic early/late promoter (EP1/LP2) and the uidA gene is under the control of the synthetic early promoter (EP2).

S-SPV-187 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 894-21.25 and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-glucuronidase (BLUOGAL AND

CPRG ASSAYS and SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of blue plaque purification was the recombinant virus designated S-SPV 187. This virus was assayed for ß-glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial 5 rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign genes.

S-SPV-187 was assayed for expression of NDV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Monoclonal mouse anti-NDV F was shown to react specifically with S-SPV-187 plaques and not with S-SPV-003 negative control plaques. All S-SPV-187 observed plaques reacted with the monoclonal antibody indicating that the virus was stably expressing the NDV foreign genes. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

S-SPV-187 is a recombinant swinepox virus expressing the NDV F protein and is useful as a vaccine in chickens against NDV infection. S-SPV-187 is also useful for expression of the NDV F protein.

059 HOMOLOGY VECTOR CONTAINING FOREIGN GENE AND UIDA: HOMOLOGY VECTOR 894-21.25 . The homology vector 894-21.25 was used to insert foreign DNA into SPV. It incorporates an E. coli ß-glucuronidase (uidA) marker gene and the NDV F gene flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the

results. Note that the ß-glucuronidase (uidA) marker gene is under the control of a synthetic early pox promoter (EP2) and the NDV F gene is under the control of a synthetic early/late pox promoter (EP1/LP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. Plasmid 894-21.22 was constructed using plasmid 847-42.7C and plasmid 493-91.11. Plasmid 847-42.7C was previously constructed by inserting the uidA gene into the unique EcoRI site within the SPV HindIII K genomic fragment. The uidA gene is under the control of the synthetic early promoter (EP2). Plasmid 493-11.1 was previously constructed and contains the EP1/LP2 promoter upstream of the coding region of the NDV F gene, an approximately 2100 base pair fragment derived from the full length molecular clone (5025F). The EP1/LP2-NDV F cassette was then inserted into a unique NotI site located upstream of the uidA gene resulting in plasmid 894-21.25. The transcriptional and translational orientation of the NDV F gene is opposite to that of the uidA gene.

S-SPV-188:

S-SPV-188 is a swinepox virus that expresses 4 foreign genes. The gene for Newcastle disease virus (NDV) HN and the gene for E. coli ß-galactosidase (lacZ) were inserted into a unique Not1 restriction site (Not1 linkers inserted into a unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment). The gene for Newcastle disease virus (NDV) F and the gene for E. coli ß-glucuronidase (uidA) were inserted into a unique Not1 site (Not1 linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment). The NDV HN gene is under the control of the synthetic early/late

promoter (EP1/LP2), the NDV F gene is under the control of the synthetic early/late promoter (EP1/LP2). The lacZ gene is under the control of the synthetic late promoter (LP1), the uidA gene is under the control of the synthetic early promoter (EP2).

S-SPV-188 was derived from S-SPV-009 (Kasza Strain), which contains the EP1/LP2-NDV HN and LP1-LacZ in the 003 site. This was accomplished utilizing the homology vector 894-21.25 and virus S-SPV-009 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-glucuronidase (BLUOGAL AND CPRG ASSAYS and SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of blue plaque purification was the recombinant virus designated S-SPV 188. This virus was assayed for ß-glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial five rounds of purification, all plaques observed were blue indicating that the virus is pure, stable, and expressing the foreign genes.

S-SPV-188 was assayed for expression of NDV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Monoclonal mouse anti-NDV F and monoclonal mouse anti-NDV HN were shown to react specifically with S-SPV-188 plaques and not with S-SPV-003 negative control plaques. All S-SPV-188 observed plaques reacted with the monoclonal antibodies indicating that the virus was stably expressing the NDV foreign genes. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

EXPRESSION IN RECOMBINANT SPV. Monoclonal mouse anti-NDV F and monoclonal mouse anti-NDV HN were shown to react specifically with S-SPV-188 plaques and not with S-SPV-003 negative control plaques. All S-SPV-188 observed plaques reacted with the monoclonal antibodies indicating that the virus was stably expressing the NDV foreign genes. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

S-SPV-188 is a recombinant swinepox virus expressing the NDV HN and F proteins and is useful as a vaccine in chickens against NDV infection. S-SPV-188 is also useful for expression of the NDV HN and F proteins.

059 HOMOLOGY VECTOR CONTAINING FOREIGN GENE AND UIDA: HOMOLOGY VECTOR 894-21.25 . The homology vector 894-21.25 was used to insert foreign DNA into SPV. It incorporates an E. coli ß-glucuronidase (uidA) marker gene and the NDV F gene flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the ß-glucuronidase (uidA) marker gene is under the control of a synthetic early pox promoter (EP2) and the NDV F gene is under the control of a synthetic early/late pox promoter (EP1/LP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. Plasmid 894-21.22 was constructed using plasmid 847-42.7C and plasmid 493-91.11. Plasmid 847-42.7C was previously constructed by inserting the uidA gene into the unique EcoRI site within the SPV HindIII K genomic fragment. The uidA gene is under the control of the synthetic

early promoter (EP2). Plasmid 493-11.1 was previously constructed and contains the EP1/LP2 promoter upstream of the coding region of the NDV F gene, an approximately 2100 base pair fragment derived from the full length molecular clone (5025F). The EP1/LP2-NDV F cassette was then inserted into a unique NotI site located upstream of the uidA gene resulting in plasmid 894-21.25. The transcriptional and translational orientation of the NDV F gene is opposite to that of the uidA gene.

S-SPV-148:

S-SPV-148 is a swinepox virus that expresses two foreign genes. The gene for bovine herpersvirus (BHV-1) truncated glycoprotein D (gD) and the gene for E. coli ß-galactosidase (lacZ) were inserted into a unique Not I restriction site (Not I linkers inserted into a unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment). The gD gene is under the control of the synthetic late/early promoter (LP2EP2) and the lacZ gene is under the control of the synthetic late promoter (LP1).

S-SPV-148 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 859-52.30 and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-148. This virus was assayed for ß-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are

blue indicating that the virus is pure, stable, and expressing the foreign genes.

To confirm the expression of the BHV-1 gD gene product, cells were infected with S-SPV-148 and samples of infected cell lysates and culture supernatients were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal bovine anti-BHV-1 serum was used to detect expression of BHV-1 specific proteins. The cell lysate from SPV-148 infected cells exhibited bands corresponding to 60 kd, which is the expected size of the BHV-1 truncated gD protein.

S-SPV-148 is a recombinant swinepox virus expressing the BHV-1 gD protein and is useful as a vaccine in cows against BHV-1 infection. S-SPV-148 is also useful for expression of the BHV-1 gD protein. Infectious bovine rhinotracheitis virus glycoprotein D (IBR gD) having a deleted transmembrane domain improves the immune response against IBR gD and improves the vaccine efficiency.

HOMOLOGY VECTOR 859-52.30. The homology vector 859-52.30 was used to insert foreign DNA into SPV. It incorporates an E. coli ß-galactosidase (lacZ) marker gene and the truncated bovine herpesvirus glycoprotein D (gD) gene flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the ß galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1) and the truncated BHV-1 gD gene is under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by

joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII fragment M (23). Fragment 2 is an approximately 1086 base pair EcoRI to BamHI fragment of the BHV-1 truncated gD gene synthesized by polymerase chain reaction (PCR) (15,42) using DNA from the BHV-1 Cooper strain genomic fragment. The upstream primer (5'- CGGAATTCACAAGGGCCGACATTGGCC -3') synthesizes from the 5' end of the BHV-1 gD gene and introduces an EcoRI site at the 5' end of the gene. The downstream primer was (5'- GCTGGGATCCACGGCGTCGGGGGCCGCGGGCGT -3') synthesizes from the 3' end of the BHV-1 gD gene, introduces an BamHI site at the 3' end of the gene. The PCR product was digested with EcoRI and BamHI to yield a fragment 1086 base pairs in length corresponding to the BHV-1 truncated gD gene. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair AccI to HindIII restriction sub-fragment of the SPV HindIII restriction fragment M (23). The AccI site in the SPV homology vector was converted to a unique NotI site using synthetic linkers.

S-SPV-186:

S-SPV-186 is a swinepox virus that expresses two foreign genes. The gene for canine distemper virus (CDV) hemagluttinin (HA) and the gene and the gene for E. coli E. coli ß-glucuronidase (uidA) were inserted into a unique Not I site (Not I linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment).

The HA gene is under the control of the synthetic late promoter (LP2), and the uidA gene is under the control of the synthetic early promoter (EP2).

S-SPV-186 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 899-20 and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß- glucuronidase (x-gluc). The final result of red plaque purification was the recombinant virus designated S-SPV-186. This virus was assayed for ß glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign genes.

To confirm the expression of the CDV HA gene product, cells were infected with S-SPV-186 and samples of infected cell lysates and culture supernatients were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal dog anti-CDV serum was used to detect expression of CDV specific protein. The cell lysate from SPV-186 infected cells exhibited bands corresponding to 70kd, which is the expected size of the CDV HA protein.

S-SPV-186 is a recombinant swinepox virus expressing the CDV HA protein and is useful as a vaccine in dogs against CDV infection. S-SPV-186 is also useful for expression of the CDV HA protein.

HOMOLOGY VECTOR 899-20. The homology vector 899-20 was used to insert foreign DNA into SPV. It incorporates an E. coli ß- glucuronidase (uidA) marker gene and the canine distemper virus (CDV) hemagluttinin (HA) gene flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the ß glucuronidase (uidA) marker gene is under the control of a synthetic early pox promoter (EP2) and the CDV HA gene is under the control of a synthetic late pox promoter (LP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), The CDV HA gene is an approximately 1875 base pair BglII fragment synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using RNA from the CDV (NVSL challenge strain). The upstream primer (5'-GAAGATCTAATGCTCTCCTACCAAGACAAGGTGGGTGCCT-3') synthesizes from the 5' end of the CDV HA gene and introduces an BglII site at the 5' end of the gene. The downstream primer was (5'-GAAGATCTTCAAGGTTTTGAACGGTCACATGAGAATCTT -3') synthesizes from the 3' end of the CDV HA gene, introduces an BglII site at the 3' end of the gene, and was used for reverse transcription and polymerase chain reaction. The PCR product was digested with BglII to yield a fragment 1875 base pairs in length corresponding to the CDV HA gene.

<u>**S-SPV-185:**</u>

S-SPV-185 is a swinepox virus that expresses five foreign genes. The gene for bovine herpesvirus BHV-1 glycoprotein D (gD) and the gene for E. coli ß-galactosidase (lacZ) were inserted into a unique Not I restriction site (Not I linkers inserted into a

unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment). The gene for bovine cytokine interleukin-12 (bIL-12) p40, p35 and the gene for E. coli _-glucuronidase (uidA) genes were inserted into a unique Not I site (Not I linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment). The gD gene is under the control of the synthetic late/early promoter (LP2EP2), The bIL-12 p40 and p35 genes are under the control of the synthetic late promoter (LP2 and LP1), the uidA gene is under the control of synthetic early promoter (EP2) and the lacZ gene is under the control of the synthetic late promoter (LP1).

S-SPV-185 was derived from S-SPV-148 (Kasza Strain). This was accomplished utilizing the homology vector 870-56 and virus S-SPV-148 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß- glucuronidase (x-gluc). The final result of red plaque purification was the recombinant virus designated S-SPV-185. This virus was assayed for ß- glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign genes.

To confirm the expression of the BHV-1 gD and bIL-12 p40, p35 gene products, cells were infected with S-SPV-185 and samples of infected cell lysates and culture supernatients were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal goat anti-hIL-12 and polyclonal bovine

anti-BHV sera were used to detect expression of bIL-12 and BHV-1 gD specific proteins. The cell lysate from SPV-185 infected cells exhibited bands corresponding to 60 kd, 40kd and 35 kd, which is the expected size of the BHV-1 truncated gD, bIL-12 p40 and p35 proteins.

S-SPV-185 was assayed for expression of bIL-12 specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal goat anti-hIL-12 serum was shown to react specifically with S-SPV-185 plaques and not with S-SPV-148 negative control plaques. All S-SPV-185 observed plaques reacted with serological reagent indicating that the virus was stably expressing the bovine foreign genes. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

S-SPV-185 is a recombinant swinepox virus expressing the bovine antigen proteins and is useful as a vaccine in cows against viral infection. S-SPV-185 is also useful for expression of the BHV-1 gD, bIL-12 p40 and p35 proteins. A vaccine containing S-SPV-185 stimulates cell mediated immunity and improves growth and weight gain of the animal.

HOMOLOGY VECTOR 870-56. The homology vector 870-56 was used to insert foreign DNA into SPV. It incorporates an E. coli ß-glucuronidase (uidA) marker gene and the bovine cytokine interleukin-12 (bIL-12) p40 and p35 genes flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the ß-glucuronidase (uidA) marker gene is under the control of a synthetic early pox

promoter (EP2) and bovine cytokine interleukin-12 genes (bIL-12) is under the control of a synthetic late pox promoters (LP2 and LP1). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. Plasmid 870-56 was constructed using plasmid 847-42.2C. This plasmid was previously constructed by inserting the uidA gene into the unique EcoRI site within the SPV HindIII K genomic fragment. The uidA gene is under the control of the synthetic early promoter (EP2). The bIL-12 genes were then inserted into a unique NotI site located upstream of the uidA gene resulting in plasmid 870-56. The transcriptional and translational orientation of the bIL-12 genes are the same as the uidA gene.

The bIL-12 p40 gene is an approximately 984 base pair BamHI to BamHI fragment synthesized by polymerase chain reaction (PCR) (15,42) using DNA from the plasmid containing IL-12 gene. The upstream primer (5'-CGTCGGATCCAATGCACCCTCAGCAGTTGGTC -3') synthesizes from the 5' end of the bIL-12 p40 gene and introduces an BamHI site at the 5' end of the gene. The downstream primer was (5'-GTTGGATCCTAACTGCAGGACACAGATGCCC-3') synthesizes from the 3' end of the bIL-12 p40 gene, introduces an BamHI site at the 3' end of the gene. The PCR product was digested with BamHI to yield a fragment 984 base pairs in length corresponding to the bIL-12 p40 gene. The bIL-12 p35 gene is an approximately 665 base pair BglII to BglII fragment synthesized by polymerase chain reaction (PCR) (15,42) using DNA from the plasmid containing IL-12 gene. The upstream primer (5'- GTCAGATCTAATGTGCCCGCTTCGCAGCCTCCTCCTCATA -3') synthesizes from the 5' end of the bIL-12 p35 gene and introduces an BglII site at the 5' end of the gene. The downstream primer was (5'-CTCAGAGATCTAGGAAGAACTCAGATAGCTCA-3') synthesizes from

the 3' end of the bIL-12 p35 gene, introduces an BglII site at the 3' end of the gene. The PCR product was digested with BgIII to yield a fragment 665 base pairs in length corresponding to the bIL-12 p35 gene.

S-SPV-184:

S-SPV-184 is a swinepox virus that expresses five foreign genes. The gene for bovine herpesvirus BHV-1 glycoprotein D (gD) and the gene for E. coli ß-galactosidase (lacZ) were inserted into a unique Not I restriction site (Not I linkers inserted into a unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment). The gene for bovine cytokine interleukin-12 (bIL-12) p40, p35 and the gene for E. coli _-glucuronidase (uidA) genes were inserted into a unique Not I site (Not I linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment). The gD gene is under the control of the synthetic late/early promoter (LP2EP2), The bIL-12 p40 gene is under the control of the synthetic late promoter (LP2), the bIL-12 p35 gene is under the control of an internal ribosomal entry site (IRES), the uidA gene is under the control of synthetic early promoter (EP2) and the lacZ gene is under the control of the synthetic late promoter (LP1).

S-SPV-184 was derived from S-SPV-148 (Kasza Strain). This was accomplished utilizing the homology vector 870-46 and virus S-SPV-148 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß- glucuronidase (x-gluc). The final result of red plaque purification was the recombinant virus designated S-SPV-184. This virus was assayed for ß- glucuronidase expression, purity, and

insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign genes.

To confirm the expression of the BHV-1 gD and bIL-12 p40, p35 gene products, cells were infected with S-SPV-184 and samples of infected cell lysates and culture supernatients were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal goat anti-hIL-12 and polyclonal bovine anti-BHV sera were used to detect expression of bIL-12 and BHV-1 gD specific proteins. The cell lysate from SPV-184 infected cells exhibited bands corresponding to 60 kd, 40kd and 35 kd, which is the expected size of the BHV-1 truncated gD, bIL-12 p40 and p35 proteins.

S-SPV-184 was assayed for expression of SIV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal goat anti-hIL-12 serum was shown to react specifically with S-SPV-184 plaques and not with S-SPV-148 negative control plaques. All S-SPV-184 observed plaques reacted with serological reagent indicating that the virus was stably expressing the bovine foreign genes. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

S-SPV-184 is a recombinant swinepox virus expressing the bovine antigen proteins and is useful as a vaccine in cows against viral infection. S-SPV-184 is also useful for expression of the BHV-1 gD, bIL-12 p40 and

p35 proteins. A vaccine containing S-SPV-184 stimulates cell mediated immunity and improves growth and weight gain of the animal.

HOMOLOGY VECTOR 870-46. HOMOLOGY VECTOR 870-46. The homology vector 870-46 was used to insert foreign DNA into SPV. It incorporates an E. coli ß-glucuronidase (uidA) marker gene and the bovine cytokine interleukin-12 (bIL-12) p40 and p35 genes flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the ß-glucuronidase (uidA) marker gene is under the control of a synthetic early pox promoter (EP2) and bovine cytokine interleukin-12 genes (bIL-12) is under the control of a synthetic late pox promoter (LP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. Plasmid 870-46 was constructed using plasmid 847-42.2C. This plasmid was previously constructed by inserting the uidA gene into the unique EcoRI site within the SPV HindIII K genomic fragment. The uidA gene is under the control of the synthetic early promoter (EP2). The bIL-12 genes were then inserted into a unique NotI site located upstream of the uidA gene resulting in plasmid 870-46. The transcriptional and translational orientation of the bIL-12 genes are the same as the uidA gene.
The bIL-12 p40 gene is an approximately 984 base pair BamHI to BamHI fragment synthesized by polymerase chain reaction (PCR) (15,42) using DNA from the plasmid containing IL-12 gene. The upstream primer (5'-CGTCGGATCCAATGCACCCTCAGCAGTTGGTC -3') synthesizes from the 5' end of the bIL-12 p40 gene and introduces an BamHI site at the 5' end of the gene. The downstream

primer was (5'-GTTGGATCCTAACTGCAGGACACAGATGCCC-3') synthesizes from the 3' end of the bIL-12 p40 gene, introduces an BamHI site at the 3' end of the gene. The PCR product was digested with BamHI to yield a fragment 984 base pairs in length corresponding to the bIL-12 p40 gene. The IRES sequence is an 509 base pairs fragment synthesized by PCR using DNA from Novagen®. The upstream primer (5'-AATGGCGCGCCGGTTATTTTCCACCATA-3') synthesized from the 5'-end of the IRES and introduces an AscI site at the 5'-end. The downstream primer was (5'- GTGGGATCCATATTATCATCGTGTTTTTC -3') synthesizes from the 3' end of the IRES, introduces an BamHI site at the 3' end of the gene. The PCR product was digested with AscI and BamHI to yield a fragment 509 base pairs in length corresponding to the IRES sequence. The bIL-12 p35 gene is an approximately 665 base pair BglII to AscI fragment synthesized by polymerase chain reaction (PCR) (15,42) using DNA from the plasmid containing IL-12 gene. The upstream primer (5'-GTCAGATCTAATGTGCCCGCTTCGCAGCCTCCTCCTCATA -3') synthesizes from the 5' end of the bIL-12 p35 gene and introduces an BglII site at the 5' end of the gene. The downstream primer was (5'- CCTGGCGCGCCTAGGAAGAACTCAGATA -3') synthesizes from the 3' end of the bIL-12 p35 gene, introduces an AscI site at the 3' end of the gene. The PCR product was digested with BgIII and AscI to yield a fragment 665 base pairs in length corresponding to the bIL-12 p35 gene.

S-SPV-183:

S-SPV-183 is a swinepox virus that expresses two foreign genes. The gene for canine distempter virus (CDV) fusion (F) and the gene for E. coli E. coli ß-glucuronidase (uidA) were inserted into a unique Not I site (Not I linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment). The F gene is

under the control of the synthetic late promoter (LP1), and the uidA gene is under the control of the synthetic early promoter (EP2).

S-SPV-183 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 888-81 and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-glucuronidase (x-gluc). The final result of blue plaque purification was the recombinant virus designated S-SPV-183. This virus was assayed for ß-glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign genes.

To confirm the expression of the CDV F gene product, cells were infected with S-SPV-183 and samples of infected cell lysates and culture supernatients were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal dog anti-CDV serum was used to detect expression of CDV specific protein. The cell lysate from SPV-183 infected cells exhibited band corresponding to 60kd, which is the expected size of the CDV F protein.

S-SPV-183 is a recombinant swinepox virus expressing the CDV F protein and is useful as a vaccine in dogs against CDV infection. S-SPV-183 is also useful for expression of the CDV F protein.

HOMOLOGY VECTOR 888-81. The homology vector 888-81 was used to insert foreign DNA into SPV. It incorporates an E. coli ß-glucuronidase (uidA) marker gene and the canine distemper virus (CDV) fusion (F) gene flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the ß-glucuronidase (uidA) marker gene is under the control of a synthetic early pox promoter (EP2) and the canine distemper virus fusion (F) gene is under the control of a synthetic late pox promoter (LP1). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. Plasmid 888-81 was constructed using plasmid 847-42.2C. This plasmid was previously constructed by inserting the uidA gene into the unique EcoRI site within the SPV HindIII K genomic fragment. The uidA gene is under the control of the synthetic early promoter (EP2). The CDV F gene was then inserted into a unique NotI site located upstream of the uidA gene resulting in plasmid 888-81. The transcriptional and translational orientation of the CDV F gene is the same as the uidA gene.

The CDV F gene is an approximately 2000 base pair BamHI fragment synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using RNA from the CDV (NVSL challenge strain). The upstream primer (5′- CGGGATCCCATGCACAGGGGAATCCCCAAAAGCTCCACC-3′) synthesizes from the 5′ end of the CDV F gene and introduces an BamHI site at the 5′ end of the gene. The downstream primer was (5′- CGGGATCCTCAGAGTGATCTCACATAGGATTTCGAAGTTC-3′) synthesizes from the 3′ end of the CDV F gene, introduces an BamHI site at the 3′ end of the gene, and

was used for reverse transcription and polymerase chain reaction. The PCR product was digested with BamHI to yield a fragment 2000 base pairs in length corresponding to the CDV F gene.

S-SPV-165:

S-SPV-165 is a swinepox virus that expresses three foreign genes. The genes for bovine cytokine interleukin-12 (IL-12) p35, p40 and the gene for E. coli ß-glucuronidase (uidA) were inserted into a unique Not I site (Not I linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment). The p40 gene is under the control of the synthetic late promoter (LP2), the p35 gene is under the control of the synthetic late promoter (LP1) and the uidA gene is under the control of the synthetic early promoter (EP2).

S-SPV-165 was derived from S-SPV-001 (Kāsza Strain). This was accomplished utilizing the homology vector 870-56 and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-glucuronidase (X-gluc). The final result of blue plaque purification was the recombinant virus designated S-SPV-165. This virus was assayed for ß-glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign genes.

To confirm the expression of the bIL-12 gene products, cells were infected with S-SPV-165 and samples of

infected cell lysates and culture supernatients were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal goat anti-human IL-12 (hIL-12) serum was used to detect expression of bIL-12 specific proteins. The cell lysate from SPV-165 infected cells exhibited bands corresponding to 40kd, and 35kd, which is the expected size of the bIL-12 p40 and p35 proteins, respectively.

S-SPV-165 was assayed for expression of IL-12 specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. A polyclonal a polyclonal goat anti-hIL-12 antiserum was shown to react specifically with S-SPV-165 plaques and not with S-SPV-001 negative control plaques. All S-SPV-165 observed plaques reacted with serological reagent indicating that the virus was stably expressing the bIL-12 foreign genes. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

S-SPV-165 is a recombinant swinepox virus expressing the bIL-12 p40 and p35 proteins and is useful as a vaccine in cows against viral infection. S-SPV-165 is also useful for expression of the bIL-12 proteins. A vaccine containing S-SPV-165 stimulates cell mediated immunity and improves growth and weight gain of the animal.

HOMOLOGY VECTOR 870-56. The homology vector 870-56 was used to insert foreign DNA into SPV. It incorporates an E. coli ß-glucuronidase (uidA) marker gene and the bovine cytokine interleukin-12 (bIL-12) p40 and p35 genes flanked by SPV DNA. When this

homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the ß-glucuronidase (uidA) marker gene is under the control of a synthetic early pox promoter (EP2) and bovine cytokine interleukin-12 genes (bIL-12) is under the control of a synthetic late pox promoters (LP2 and LP1). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. Plasmid 870-56 was constructed using plasmid 847-42.2C. This plasmid was previously constructed by inserting the uidA gene into the unique EcoRI site within the SPV HindIII K genomic fragment. The uidA gene is under the control of the synthetic early promoter (EP2). The bIL-12 genes were then inserted into a unique NotI site located upstream of the uidA gene resulting in plasmid 870-56. The transcriptional and translational orientation of the bIL-12 genes are the same as the uidA gene.

The bIL-12 p40 gene is an approximately 984 base pair BamHI to BamHI fragment synthesized by polymerase chain reaction (PCR) (15,42) using DNA from the plasmid containing IL-12 gene. The upstream primer (5'-CGTCGGATCCAATGCACCCTCAGCAGTTGGTC -3') synthesizes from the 5' end of the bIL-12 p40 gene and introduces an BamHI site at the 5' end of the gene. The downstream primer was (5'-GTTGGATCCTAACTGCAGGACACAGATGCCC-3') synthesizes from the 3' end of the bIL-12 p40 gene, introduces an BamHI site at the 3' end of the gene. The PCR product was digested with BamHI to yield a fragment 984 base pairs in length corresponding to the bIL-12 p40 gene. The bIL-12 p35 gene is an approximately 665 base pair BglII to BglII fragment synthesized by polymerase chain reaction (PCR) (15,42) using DNA from the plasmid containing IL-12 gene. The upstream primer

(5'- GTCAGATCTAATGTGCCCGCTTCGCAGCCTCCTCCTCATA -3') synthesizes from the 5' end of the bIL-12 p35 gene and introduces an BglII site at the 5' end of the gene. The downstream primer was (5'-CTCAGAGATCTAGGAAGAACTCAGATAGCTCA-3') synthesizes from the 3' end of the bIL-12 p35 gene, introduces an BglII site at the 3' end of the gene. The PCR product was digested with BgIII to yield a fragment 665 base pairs in length corresponding to the bIL-12 p35 gene.

S-SPV-164:

S-SPV-164 is a swinepox virus that expresses three foreign genes. The genes for bovine cytokine interleukin-12 (IL-12) p35, p40 and the gene for E. coli ß-glucuronidase (uidA) were inserted into a unique Not I site (Not I linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment). The p40 gene is under the control of the synthetic late promoter (LP2), the p35 gene is under the control of an internal ribosomal entry site (IRES), and the uidA gene is under the control of the synthetic early promoter (EP2).

S-SPV-164 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 870-46 and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-glucuronidase (X-gluc). The final result of blue plaque purification was the recombinant virus designated S-SPV-164. This virus was assayed for ß-glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of

purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign genes.

To confirm the expression of the bIL-12 gene products, cells were infected with S-SPV-164 and samples of infected cell lysates and culture supernatients were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal goat anti-human IL-12 (hIL-12) serum was used to detect expression of bIL-12 specific proteins. The cell lysate from SPV-164 infected cells exhibited bands corresponding to 40kd, and 35kd, which is the expected size of the bIL-12 p40 and p35 proteins, respectively.

S-SPV-164 was assayed for expression of IL-12 specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. A polyclonal a polyclonal goat anti-hIL-12 antiserum was shown to react specifically with S-SPV-164 plaques and not with S-SPV-001 negative control plaques. All S-SPV-164 observed plaques reacted with serological reagent indicating that the virus was stably expressing the bIL-12 foreign genes. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

S-SPV-164 is a recombinant swinepox virus expressing the bIL-12 p40 and p35 proteins and is useful as a vaccine in cows against viral infection. S-SPV-164 is also useful for expression of the bIL-12 proteins. A vaccine containing S-SPV-164 stimulates cell mediated immunity and improves growth and weight gain of the animal.

HOMOLOGY VECTOR 870-46. The homology vector 870-46 was used to insert foreign DNA into SPV. It incorporates an E. coli ß-glucuronidase (uidA) marker gene and the bovine cytokine interleukin-12 (bIL-12) p40 and p35 genes flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the ß-glucuronidase (uidA) marker gene is under the control of a synthetic early pox promoter (EP2) and bovine cytokine interleukin-12 genes (bIL-12) is under the control of a synthetic late pox promoter (LP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. Plasmid 870-46 was constructed using plasmid 847-42.2C. This plasmid was previously constructed by inserting the uidA gene into the unique EcoRI site within the SPV HindIII K genomic fragment. The uidA gene is under the control of the synthetic early promoter (EP2). The bIL-12 genes were then inserted into a unique NotI site located upstream of the uidA gene resulting in plasmid 870-46. The transcriptional and translational orientation of the bIL-12 genes are the same as the uidA gene.

The bIL-12 p40 gene is an approximately 984 base pair BamHI to BamHI fragment synthesized by polymerase chain reaction (PCR) (15,42) using DNA from the plasmid containing IL-12 gene. The upstream primer (5'-CGTCGGATCCAATGCACCCTCAGCAGTTGGTC -3') synthesizes from the 5' end of the bIL-12 p40 gene and introduces an BamHI site at the 5' end of the gene. The downstream primer was (5'-GTTGGATCCTAACTGCAGGACACAGATGCCC-3') synthesizes from the 3' end of the bIL-12 p40 gene, introduces an BamHI site at the 3' end of the gene. The PCR product was digested with BamHI to yield a fragment

984 base pairs in length corresponding to the bIL-12 p40 gene. The IRES sequence is an 509 base pairs fragment synthesized by PCR using DNA from Novagen®. The upstream primer (5'-AATGGCGCGCCGGTTATTTTCCACCATA-3') synthesized from the 5'-end of the IRES and introduces an AscI site at the 5'-end. The downstream primer was (5'- GTGGGATCCATATTATCATCGTGTTTTTC -3') synthesizes from the 3' end of the IRES, introduces an BamHI site at the 3' end of the gene. The PCR product was digested with AscI and BamHI to yield a fragment 509 base pairs in length corresponding to the IRES sequence. The bIL-12 p35 gene is an approximately 665 base pair BglII to AscI fragment synthesized by polymerase chain reaction (PCR) (15,42) using DNA from the plasmid containing IL-12 gene. The upstream primer (5'-GTCAGATCTAATGTGCCCGCTTCGCAGCCTCCTCCTCATA -3') synthesizes from the 5' end of the bIL-12 p35 gene and introduces an BglII site at the 5' end of the gene. The downstream primer was (5'- CCTGGCGCGCCTAGGAAGAACTCAGATA -3') synthesizes from the 3' end of the bIL-12 p35 gene, introduces an AscI site at the 3' end of the gene. The PCR product was digested with BgIII and AscI to yield a fragment 665 base pairs in length corresponding to the bIL-12 p35 gene.

S-SPV-176 :

S-SPV-176 is a swinepox virus that expresses four foreign genes. The gene for bovine respiratory syncytial virus glycoprotein (G) and the gene for E. coli ß-galactosidase (lacZ) were inserted into a unique Not I restriction site (NotI linkers inserted into a unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment). The gene for bovine respiratory syncytial virus fusion (F) and the gene for E. coli ß-glucuronidase (uidA) were inserted into a unique NotI site (NotI linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region

of the 6.7 kb SPV HindIII K fragment). . The gene for bovine respiratory syncytial virus glycoprotein G is under the control of the synthetic late/early promoter (LP2EP2), . The gene for bovine respiratory syncytial virus fusion (F) is under the control of the synthetic late promoter (LP1). The lacZ gene is under the control of the synthetic late promoter (LP1), the uidA gene is under the control of the synthetic early promoter (EP2).

S-SPV-176 was derived from S-SPV-020 (Kasza Strain). This was accomplished utilizing the homology vector 888-38.9 and virus S-SPV-020 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ß-glucuronidase (SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of blue plaque purification was the recombinant virus designated S-SPV-176 . This virus was assayed for ß-galactosidase and ß-glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign genes.

To confirm the expression of bovine respiratory syncytial virus glycoprotein (G) and fusion (F) gene products, cells were infected with S-SPV-176 and samples of infected cell lysates were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. Bovine anti-FITC (Accurate Chemicals) or a F specific Mab (Mab19) was used to detect expression of BRSV specific proteins. The cell lysate from SPV-176 infected cells exhibited bands corresponding to 80 kd

to 90 kd and 70 kd which is the expected size of the bovine respiratory syncytial virus glycoprotein (G) and fusion (F) gene products. The assay described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

S-SPV-176 is a recombinant swinepox virus expressing the PRRS ORF5 and ORF6 proteins and is useful as a vaccine in swine against PRRS infection. S-SPV-134 is also useful for expression of the PRRS ORF5 and ORF6 proteins.

HOMOLOGY VECTOR 888-38.9. The homology vector 888-38.9 was used to insert foreign DNA into SPV. It incorporates an E. coli ß-glucuronidase (uidA) marker gene and the bovine respiratory syncytial virus fusion (F) gene flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the ß-glucuronidase (uidA) marker gene is under the control of a synthetic early pox promoter (EP2) and the bovine respiratory syncytial virus fusion (F) gene is under the control of a synthetic late pox promoter (LP1). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. Plasmid 888-38.9 was constructed using plasmid 847-42.7C. This plasmid was previously constructed by inserting the uidA gene into the unique EcoRI site within the SPV HindIII K genomic fragment. The uidA gene is under the control of the synthetic early promoter (EP2). The bovine respiratory syncytial virus fusion (F) gene was then inserted into a unique

downstream primer (5'-CTCTGGATCCTACAGCCATGAGGATGATCATCAGC-3') synthesizes from the 3' end of the fusion (F) gene, introduces an BamHI site at the 3' end of the gene, and was used for reverse transcription and polymerase chain reaction. The PCR product was digested with Bam HI to yield a fragment 1723 base pairs in length corresponding to the fusion (F) gene.

S-SPV-143:

S-SPV-143 is a swinepox virus that expresses three foreign genes. The genes for bovine viral diarrhea virus type 1 (BVDV1) E2 glycoprotein and the gene for E. coli ß-galactosidase (lacZ) were inserted into a unique Not I restriction site (Not I linkers inserted into a unique AccI restriction site in the OIL ORF of the SPV HindIII M fragment). The genes for bovine viral diarrhea d virus type 1(BVDV1) E glycoprotein, bovine viral diarrhea virus type 2(BVDV2) E2 gylcoprotein and the E. coli ß-glucuronidase (uidA) marker gene were inserted into unique Bam HI, Not I and Pst I site respectively (sites originating from a synthetic polylinker inserted into a unique EcoRI restriction site within an approximately 3.2 kb region (SEQ ID NO) of the 6.7 kb SPV HindIII K Fragment). The E gene and the lacZ gene are under the control of the synthetic late promoter (LP1), and the uidA gene is under the control of the synthetic early promoter.

S-SPV-143 was derived from S-SPV-051 (Kasza Strain). This was accomplishing utilizing the homology vector 874-06.20B and virus S-SPV-051 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The BVDV type 1 E2 gene and the lacZ gene in the HindIII M site were already present in virus S-SPV-051. The transfection stock was screened by the SCREEN FOR

RECOMBINANT SPV EXPRESSING ß-GLUCURONIDASE (x-Gluc ASSAY and SCREEN FROM RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of green plaque purification was the recombinant virus designated S-SPV-143. The virus was assayed for ß-glucuronidase expression, purity, and insert stability by multiple passages monitored by the green plaque assay as describes in Materials and Methods. After the initial four rounds of purification, all plaques observed are green indicating that the virus is pure stable, and expressing the foreign genes.

To comfirm the expression of the BVDV type 2 E2 and type 1 E gene products, cells were infected with S-SPV-143 and samples of infected cell lysates were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A monoclonal mouse anti-E2 (type 2) of a monoclonal mouse anti-E (type 1)serum was used to detect expression of BVDV-specific proteins. The cell lysate from S-SPV-143 infected cell exhibited bands corresponding to 53 kd and 42 kd, which is the expected size of the E2 glycoprotein (53 kd) but slightly smaller than the expected size of the E glycoprotein (48 kd).

S-SPV-143 was assayed for expression of BVDV-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSING IN RECOMBINANT SPV. Monoclonal mouse anti-E2 sera were shown to react specifically with S-SPV-143 plaques and not with S-SPV-001 negative control plaques. All S-SPV-143 observed plaques reacted with both a type 1 E2 and a type 2 E2 serum indicating that the virus was stably expressing the BVDV E2 foreign genes. No reagent is currently available that reacts specifically with the E glycoprotein in a black plaque assay. The assays described here were carried out in

ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production for SPV recombinant vaccines.

S-S.V.-143 is a recombinant swinepox virus expressing the types 1 & 1 BVDV E2 glycoproteins and the type 1 E glycoprotein and is useful as a vaccine against BVDV infection.

HOMOLOGY VECTOR 874-06.20B. The homology vector 874-06.20B was used to insert foreign DNA into SPV. It incorporates an E. coli ß- glucuronidase (uidA) marker gene and the bovine viral diarrhea virus (BVDV) type 1 E gene and the BVDV type 2 E2 gen flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note the ß-glucuronidase marker gene is under the control of a synthetic early pox promoter (EP2), the E gene is under control of the late synthetic pox promoter (LP1) and the BVDV E2 gene is under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments form the following sources with the appropriate synthetic DNA sequences. Plasmid 874-06.20B was constructed using plasmid 847-90.2B. This plasmid was previously constructed by inserting the BVDV type 2 E2 gene under the control of the synthetic late/early promoter (LP2EP2) into a unique Not I site flanked by the EP2-uidA cassette downstream of the Not I site. The Not I originated from a synthetic polylinker inserted into the unique Eco RI site of the SPV HindIII K genomic fragment. The EP 2-uidA cassette had previously been inserted into the blunt-ended resulting in plasmid 874-

06.20B. The translational orientation of the E gene is the same as the E 2 and uidA genes.

The BVDV E gene is an approximately 744 base pair (63 bp signal sequence +681 bp coding sequence) Eco RI to Bam HI fragment synthesized by reverse transcription (RT) and polymerase chain reaction using RNA from the BVDV1-Singer strain. The upstream primer (5'-CCATGAATTCGCTGGAAAAAGCATTGCTGGCATGGGC-3'; 8/96.3) synthesizes from the 5' end of the BVDV E gene signal sequence and introduces an Eco RI site at the 5' end of the gene. The downstream primer (5'-TTCGGATCCTTACGCGTATGCTCCAAACCACGT-3'; 8/96.4) synthesizes form the 3' end of the E gene and introduces a Bam HI site at the 3' end of the gene. The PCR product was disgested with Eco RI and Bam HI to yield a fragment 744 base pairs in length corresponding to the BVDV1 E gene.

REFERENCES:

1. C. Bertholet, *et al.*, *EMBO Journal* **5**, 1951-1957 (1986).

2. R. A. Bhat, *et al.*, *Nucleic Acids Research* **17**, 1159-1176 (1989).

3. D. A. Boyden, *et al.*, *Infection and Immunity* **57**, 3808-3815 (1989).

4. D. B. Boyle and B. E. H. Coupar, *Virus Research* **10**, 343-356 (1988).

5. R. M. Buller, *et al.*, *Nature* **317**, 813-815 (1985).

6. K. J. Cremer, *et al.*, *Science* **228**, 737-739 (1985).

7. A. J. Davidson and B. Moss, *J. Mol. Biol.* **210**, 749-769 (1989).

8. A. J. Davidson and B. Moss, *J. Mol. Biol.* **210**, 771-784 (1989).

9. P. L. Earl, *et al.*, *Journal of Virology* **64**, 2448-2451 (1990).

10. J. J. Esposito, *et al.*, *Virology* **165**, 313 (1988).

11. F. A. Ferrari, *et al.*, *J. of Bacteriology* **161**, 556-562 (1985).

12. C. Flexner, *et al.*, *Vaccine* **8**, 17-21 (1990).

13. S. J. Goebel, *et al.*, *Virology* **179**, 247-266 (1990).

14. U. Gubler and B. J. Hoffman, *Gene* **25**, 263-269 (1983).

15. M. A. Innis, *et al.*, *PCR Protocols A Guide to Methods and Applications*, 84-91, Academic Press, Inc., San Diego (1990).

16. S. Joshi, *et al.*, *Journal of Virology*. **65**, 5524-5530 (1991).

17. L. Kasza, *et al.*, *Am. J. Vet. Res.* **21**, 269-273 (1960).

18. L. Kasza, Diseases of Swine, 254-260, Ed. A.D. Leman, *et al.*, The Iowa State University Press, Ames, Iowa (1981).

19. B. G. Klupp and T. C. Mettenleiter, *Virology* **182**, 732-741 (1991).

20. U. K. Laemnli, *Nature* **227**, 680-685 (1970).

21. B. Lominiczi, *et al.*, *Journal of Virology* **49**, 970-979 (1984).

22. T. Maniatis, *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1982).

23. R. F. Massung, and R. W. Moyer, *Virology* **180**, 347-354 (1991).

24. R. F. Massung, and R. W. Moyer, *Virology* **180**, 355-364 (1991).

25. B. Moss, *Science* **252**, 1662-1667 (1991).

26. E. A. Petrovskis, *et al.*, *Journal of Virology* **59**, 216-223 (1986).

27. A. K. Robbins *et al.*, *Journal of Virology* **58**, 339-347 (1986).

28. A. K. Robbins *et al.*, *Journal of Virology* **61**, 2691-2701 (1987).

29. A. C. R. Samson, *Journal of Virology* **67**, 1199-1203 (1986).

30. J. Sambrook, *et al.*, *Molecular Cloning A Laboratory Manual Second Edition*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1989).

31. Sheffy, et al., *Proceedings 65th Annual Meeting of the United States*

Livestock Association **65**, 347-353 (1961).

32. W. M. Schnitzlein and D. N. Tripathy, *Virology* **181**, 727-732, (1991).

33. J. Taylor, *et al.*, *Vaccine* **9**, 190-193, (1991).

34. M. Wachsman, *et al.*, *Journal of General Virology* **70**, 2513-2520 (1989).

35. M. W. Wathen, *et al.*, *Journal of Virology* **51**, 57-62 (1984).

36. M. Weerasinge, *Journal of Virology* **65**, 5531-5534 (1991).

37. T. Ben-Porat, *et al.*, *Journal of Virology*, volume 154, 325-334 (1986).

38. F. Zuckerman, *et al.*, *Vaccination and Control of Adjesky's Disease*, J. T. Van Oirchot (ed.). Kluwer Academic Publishers, London, pp. 107-117 (1989).

39. Paolette, *et al.*, *Journal of Virology*, volume 66, pp. 3424-3434 (June, 1992).

40. M. W. Mellencamp, *et al.*, *Journal of Clinical Microbiology*, volume 27, pp. 2208-2213 (1989).

41. L. A. Herzenberg, *et al.*, *Selected Methods in Cellular Immunology*, Freeman Publ. Co., San Francisco, 351-372 (1980).

42. Katz *et al.*, *Journal of Virology* **64**, 1808-1811 (1990).

43. Taniguchi, T., *et al.*, *Biochem. Biophys. Res. Commun.* **115** 1040-1047 (1983).

44. Cochran, M.D. and Macdonald, R.D., WO 93/02104, published February 4, 1993.

45. Galibert, F., *et al.*, *Nature* **281**, 646-650 (1979).

46. Thomsen, D.R., *et al.*, *Proc. Natl. Acad. Sci. USA* **81**, 659-663 (1984).

47. Catalog Number 267402, Beckman Instruments, Inc., Fullerton California.

48. Whalley, J.M., *et al.*, *Journal of General Virology* **57** 307-323 (1981).

49. Collett, M.S., *et al.*, *Virology* **165** 200-208 (1988).

50. Schodel, F. *et al.*, *Journal of Virology* **66**, 106-114 (1992).

51. Cochran, M.D., WO 93/25665, published December 23, 1993.

52. C.A. Hjerpe, *The bovine Respiratory Disease Complex*. Ed. by J.L. Howard, Philadelphia, W.B. Saunders Co., 670-680 (1986).

53. F. Fenner, *et al.*, *Veterinary Virology*. Academic Press, Inc., Orlando Florida, 183-202 (1987).

54. A. Leutz, et al., EMBO Journal **8**: 175-182 (1989).

55. M.J. Sekellick, et al., Journal of Interferon Research **14**: 71-79 (1994).

56. S.J. Child, et al., Virology **174**: 625-629 (1990).

57. G.P. Johnson, et al. Virology **196**: 381-401 (1993).

58. R.F Massung, et al. Virology **201**: 215-240 (1994).

60. Child, S.J. et al., Virology **174**, 625-629 (1990).

61. T.R. Phillips, et al., J. Virology **64**, 4605-4613 (1990).

62. Digby, M.R., and Lowenthal, J.W., Journal of Interferon and Cytokine Research **15**: 939 (1996).

63. G.J. Winston et al., Proc. Natl. Acad. Sci. USA **90**: 1272 (1993).

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANTS: Cochran Ph.D., Mark D
Junker M.S., David E

(ii) TITLE OF INVENTION: Recombinant Swinepox Virus

(iii) NUMBER OF SEQUENCES: 101

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: John P. White
(B) STREET: 1185 Avenue of the Americas
(C) CITY: New York
(D) STATE: New York
(E) COUNTRY: USA
(F) ZIP: 10036

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: White, John P
(B) REGISTRATION NO:28,678

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (212)278-0400
(B) TELEFAX: (212)391-0526
(C) TELEX: 422523

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3164 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox virus
(B) STRAIN: Kasza Strain

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..231

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 339..1628

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1683..3161

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
AAG CTT CTC AAT TAT GAT AAT TTT TTA AGA TTA AAA AAT TTA GTA ATG        48
Lys Leu Leu Asn Tyr Asp Asn Phe Leu Arg Leu Lys Asn Leu Val Met
 1               5                  10                  15

TAT GGA TCA CAT ATA GAA AAT ATT ATC AAA AAT ACA TAT ATG TAT TAT        96
Tyr Gly Ser His Ile Glu Asn Ile Ile Lys Asn Thr Tyr Met Tyr Tyr
            20                  25                  30

TCT AAC ATT GAT AAA GCG ATT TAT GTA ATT ATG AAG CAC TGC AAG AAA       144
Ser Asn Ile Asp Lys Ala Ile Tyr Val Ile Met Lys His Cys Lys Lys
        35                  40                  45

CAT AGT TAC TGG ATG AGG ATT CCT ATA GAA ATA CAA CGA TAT ATA TTA
    192

His Ser Tyr Trp Met Arg Ile Pro Ile Glu Ile Gln Arg Tyr Ile Leu
    50                  55                  60

TTA CAT TTA ACA ATG AAG GAC TTA TCA ATA ATA CTT AAG TAATAATGTC        241
Leu His Leu Thr Met Lys Asp Leu Ser Ile Ile Leu Lys
65                  70                  75

ATAATATTGA AAAAAAATTT TTTTTCTAGT AATGTGGCTA TTATTAGTAG CCCATGAATA     301

CATTTTGGTT ATCGTTTAAA TAGTTTGTAA GAAGGAA ATG GAT AAT ATA AGA AGA      356
                                         Met Asp Asn Ile Arg Arg
                                          1               5

ATA ATA TCA AAT ATA AAA CAG GAT GAT AAT ATA GCC ACT GAT ATG TTA       404
Ile Ile Ser Asn Ile Lys Gln Asp Asp Asn Ile Ala Thr Asp Met Leu
            10                  15                  20

GCT ACA TTT TTA AGT TCA TCG TTG CAC GTA TTT AAA TTA AAA GAG TTG       452
Ala Thr Phe Leu Ser Ser Ser Leu His Val Phe Lys Leu Lys Glu Leu
        25                  30                  35

AAA GAA ATT GTA TTA TTA CTG CTT AAT AAA GGT GCT AAT TTA AAT GGG       500
Lys Glu Ile Val Leu Leu Leu Leu Asn Lys Gly Ala Asn Leu Asn Gly
    40                  45                  50

ATA TCT ATA TAT GAT AAA ACA CCA TTT CAT TGT TAT TTT ACA TTT AAT       548
Ile Ser Ile Tyr Asp Lys Thr Pro Phe His Cys Tyr Phe Thr Phe Asn
55                  60                  65                  70

ACG AAT GTT ACA ATT AAA GTA ATA AAG TTT CTT ATT TAT CAT GGT GGT       596
Thr Asn Val Thr Ile Lys Val Ile Lys Phe Leu Ile Tyr His Gly Gly
                75                  80                  85

GAC ATT AAC AGT GTA CAT AGA TGT GGA GAC ACC ATA TTG CAT AAA TAC       644
Asp Ile Asn Ser Val His Arg Cys Gly Asp Thr Ile Leu His Lys Tyr
            90                  95                 100

CTT GGT AAT GAG AAT ATA GAT TAT AAA GTT GTT GAG TTT TTA ATA AGA       692
Leu Gly Asn Glu Asn Ile Asp Tyr Lys Val Val Glu Phe Leu Ile Arg
        105                 110                 115

AAA GGA TTT GAT GTA TGT AAA CTA AAT AAT AGT CTG AAG AAT CCT ATT       740
Lys Gly Phe Asp Val Cys Lys Leu Asn Asn Ser Leu Lys Asn Pro Ile
    120                 125                 130
```

CAT ATA TTT ACA ATT AGA CAC ATC AAT AAC ACT AAT TTA AAT ATA TTG 788
His Ile Phe Thr Ile Arg His Ile Asn Asn Thr Asn Leu Asn Ile Leu
135 140 145 150

AAT TTG CTT TGT TCG CAT ATA AAA CAT GAA TAT AAT AAA AAT GAT GAA 836
Asn Leu Leu Cys Ser His Ile Lys His Glu Tyr Asn Lys Asn Asp Glu
155 160 165

ATG ATG TCG ATA TTA AAC ACG ATG TTA AAC TAT TGT CAC GAC GAT TAT 884
Met Met Ser Ile Leu Asn Thr Met Leu Asn Tyr Cys His Asp Asp Tyr
170 175 180

ACA TGT TTT TCG GCG GTC CCA TAT ACT ATA GAT ATC ACA ACC ATA AAC 932
Thr Cys Phe Ser Ala Val Pro Tyr Thr Ile Asp Ile Thr Thr Ile Asn
185 190 195

TAT AGA GAT AAA TTA GGA TAT TCT CCT GTT GTG TAT GCA TCT ACC ACG 980
Tyr Arg Asp Lys Leu Gly Tyr Ser Pro Val Val Tyr Ala Ser Thr Thr
200 205 210

GAT AAA ACT ATC TTG GTG GAT TAT CTT ATT AAA TTA GGA GCA AAC ATG 1028
Asp Lys Thr Ile Leu Val Asp Tyr Leu Ile Lys Leu Gly Ala Asn Met
215 220 225 230

AAC ATA ACA ACG AAC GAT GGT AAT ACA TGT GGT TCG TTT GCT GTA ATG 1076
Asn Ile Thr Thr Asn Asp Gly Asn Thr Cys Gly Ser Phe Ala Val Met
235 240 245

AAT TGT AAC AGG GAT ATT AAT AGA CTA TTT CTT AAT CAA AAT CCA AAT 1124
Asn Cys Asn Arg Asp Ile Asn Arg Leu Phe Leu Asn Gln Asn Pro Asn
250 255 260

ATA GAA ACT ATA TAT AAT ACA TTG AAG ATA TTA TCG GAG AAT ATA GTA 1172
Ile Glu Thr Ile Tyr Asn Thr Leu Lys Ile Leu Ser Glu Asn Ile Val
265 270 275

TTC ATA GAC GGA TGT GAT GTA CGT ACG AAT ATG GTT AAA AAA ATA CTA 1220
Phe Ile Asp Gly Cys Asp Val Arg Thr Asn Met Val Lys Lys Ile Leu
280 285 290

ATG TAC GGA TTT ACT TTA GAT CCA CTA TTT TAC AAG AAC CAC GAT ATC 1268
Met Tyr Gly Phe Thr Leu Asp Pro Leu Phe Tyr Lys Asn His Asp Ile
295 300 305 310

ATT GTT GAA TAT TTT TCA AGT AGT ATT AAA AAG TAT AAT AAG ATT ATT 1316
Ile Val Glu Tyr Phe Ser Ser Ser Ile Lys Lys Tyr Asn Lys Ile Ile
315 320 325

TTA CAA ATG ATC GAT GAG AAA ATT GGG AAT AGA TCC GTA TAC GAT ATT 1364
Leu Gln Met Ile Asp Glu Lys Ile Gly Asn Arg Ser Val Tyr Asp Ile
330 335 340

ATA TTT ACT AAA TCA AAT ACA GGT ATG GAT GTT AGA TAT GTA TGT AAT 1412
Ile Phe Thr Lys Ser Asn Thr Gly Met Asp Val Arg Tyr Val Cys Asn
345 350 355

GAT ATC ATT ATA AAA TAT GCA AGT GTT AAA TAT TAT GGA TCT TTA ATA 1460
Asp Ile Ile Ile Lys Tyr Ala Ser Val Lys Tyr Tyr Gly Ser Leu Ile
360 365 370

AAA CGT TTG ATA TAT CAT TCT AAG AAA AGG AAG CGA AAT ATA TTA AAA 1508
Lys Arg Leu Ile Tyr His Ser Lys Lys Arg Lys Arg Asn Ile Leu Lys
375 380 385 390

GCT ATA CAT GCG ATG GAG AAT AAC ACA ACC TTG TGG AAT TAC CTA CCA 1556
Ala Ile His Ala Met Glu Asn Asn Thr Thr Leu Trp Asn Tyr Leu Pro
395 400 405

```
TTG GAA GTA AAA ATG TAT ATT ATG GAT TTC TTA CCC GAT ACT GAT ATA      1604
Leu Glu Val Lys Met Tyr Ile Met Asp Phe Leu Pro Asp Thr Asp Ile
            410                 415                 420

ACT AAC ATT CTT TTT ATG AAA AAA TGAAAATATA TACATAAGAC AGGGAATTCC     1658
Thr Asn Ile Leu Phe Met Lys Lys
        425                 430

TATTGTTTTT TTATATAGGG GAAA ATG GAT AAT CTA TAC CGA TAT ATT ACT      1709
                           Met Asp Asn Leu Tyr Arg Tyr Ile Thr
                             1               5

GTA TCC GAT ACA GTG GAC GTA GAT AAT GTA AGA AAA TTA TTA TCT TCG      1757
Val Ser Asp Thr Val Asp Val Asp Asn Val Arg Lys Leu Leu Ser Ser
 10                  15                  20                  25

TGT AAT ATC GAC GTA GTC TCT ACA ATA TTT CAA AAA TAT CTT CAT AGA      1805
Cys Asn Ile Asp Val Val Ser Thr Ile Phe Gln Lys Tyr Leu His Arg
                30                  35                  40

AAC GAT ATT AAA TTA GAT ATC GTT GAA GAG TTT GTG AAT AAC GGA GCT      1853
Asn Asp Ile Lys Leu Asp Ile Val Glu Glu Phe Val Asn Asn Gly Ala
            45                  50                  55

AAA CTG AAT GGG AAA GAT TTT AAC GAT AAA AAT ATA CCA TTG TGT ACA      1901
Lys Leu Asn Gly Lys Asp Phe Asn Asp Lys Asn Ile Pro Leu Cys Thr
        60                  65                  70

TTA TTA TCT AAT AAA TTC ATA GAT TAT AAT AGT GCC ATC GAT ATA ACA      1949
Leu Leu Ser Asn Lys Phe Ile Asp Tyr Asn Ser Ala Ile Asp Ile Thr
    75                  80                  85

AGT TTT ATG ATT ACA CAT GGA GCG GAT ATA AAT AAG AGA AAT AAG GAT      1997
Ser Phe Met Ile Thr His Gly Ala Asp Ile Asn Lys Arg Asn Lys Asp
 90                  95                 100                 105

GGG CGT ACT CCT ATA TTT TGT TTA CTA CAT AAT TCT ACA TTA AAT AAT      2045
Gly Arg Thr Pro Ile Phe Cys Leu Leu His Asn Ser Thr Leu Asn Asn
                110                 115                 120

TTA GAA TTT GTA TCT TTT ATG ATA GAC CAT GGT GCA GAT ATT ACA ATA      2093
Leu Glu Phe Val Ser Phe Met Ile Asp His Gly Ala Asp Ile Thr Ile
            125                 130                 135

GTT GAT GGA TTC GGG TTC ACA TCA TTA CAA ATA TAT TTA CAA TCA TCA      2141
Val Asp Gly Phe Gly Phe Thr Ser Leu Gln Ile Tyr Leu Gln Ser Ser
        140                 145                 150

AAT GTA CAA TTA GAT TTG GTT GAG TTA TTG ATA CAA AAG GGG GTC GAT      2189
Asn Val Gln Leu Asp Leu Val Glu Leu Leu Ile Gln Lys Gly Val Asp
    155                 160                 165

GTA AAT ATA CAT AAT AAT TGG TTC TAT TAC AAT ACA TTA CAT TGT TAT      2237
Val Asn Ile His Asn Asn Trp Phe Tyr Tyr Asn Thr Leu His Cys Tyr
170                 175                 180                 185

ATA AAG AAA AAT TAT AAC CGT ATT AAT ATG GAT ATT ATA AAA TAT ATA      2285
Ile Lys Lys Asn Tyr Asn Arg Ile Asn Met Asp Ile Ile Lys Tyr Ile
                190                 195                 200

ATG GAC AAT GGA TTT ACA ATT AAT GAG AAT AAA TTT ACC AAA TCA ACA      2333
Met Asp Asn Gly Phe Thr Ile Asn Glu Asn Lys Phe Thr Lys Ser Thr
            205                 210                 215

TTT TTA GAT ATA TTG GTA TCA ATT ATT GAT AGT AAA AAC TTT GAC TCA      2381
Phe Leu Asp Ile Leu Val Ser Ile Ile Asp Ser Lys Asn Phe Asp Ser
        220                 225                 230
```

```
AAC GTT GTT GAT TTT ATA TTA AAA TAT ATT GAT ATT AAT GAA AAG AAT     2429
Asn Val Val Asp Phe Ile Leu Lys Tyr Ile Asp Ile Asn Glu Lys Asn
    235                 240                 245

ATT TTT GAT TTT ACG CCA TTA TAC TGT TCT GTA GAT GCA AAT AAT GAA     2477
Ile Phe Asp Phe Thr Pro Leu Tyr Cys Ser Val Asp Ala Asn Asn Glu
250                 255                 260                 265

AAG ATG TGT TCT TAT TTA CTA AAA AAG AAT GCA GAC CCT AAT ATT ATC     2525
Lys Met Cys Ser Tyr Leu Leu Lys Lys Asn Ala Asp Pro Asn Ile Ile
                270                 275                 280

ACA GTA TTT GGT GAA ACG TGT ATA CTA ACA GCT ATC AAT AAT CAT AAT     2573
Thr Val Phe Gly Glu Thr Cys Ile Leu Thr Ala Ile Asn Asn His Asn
            285                 290                 295

AAA AAT ATA TTA TAT AAA CTA TTA AAT TAT GAT ATA GAT ATA AAT ACT     2621
Lys Asn Ile Leu Tyr Lys Leu Leu Asn Tyr Asp Ile Asp Ile Asn Thr
        300                 305                 310

ATC CAA AAT ACA TTA TTT AAA CTG GAA CAA GAT ATT ATT AAC TCT ACC     2669
Ile Gln Asn Thr Leu Phe Lys Leu Glu Gln Asp Ile Ile Asn Ser Thr
    315                 320                 325

ATA GAT ACT TAC TAT TAC AAT AAT CTT GTT AAA AAA GAA CAT TTT ATA     2717
Ile Asp Thr Tyr Tyr Tyr Asn Asn Leu Val Lys Lys Glu His Phe Ile
330                 335                 340                 345

AAA TTA TTT CTA GCC TAC ATA GTT AAG AGG TAT GAA AAA AAT ATA GGA     2765
Lys Leu Phe Leu Ala Tyr Ile Val Lys Arg Tyr Glu Lys Asn Ile Gly
                350                 355                 360

ATA TTA TTT CTT GAT TAT CCC ACT CTT GGT GAA TAT TTC GTG AAA TTT     2813
Ile Leu Phe Leu Asp Tyr Pro Thr Leu Gly Glu Tyr Phe Val Lys Phe
            365                 370                 375

ATA GAT ACG TGT ATG ATG GAA ATA TTT GAG ATG AAA TCA GAT AAG GCT     2861
Ile Asp Thr Cys Met Met Glu Ile Phe Glu Met Lys Ser Asp Lys Ala
        380                 385                 390

GGT AAT ACG GAT ATA TAT TCT ATT ATA TTT ACG AAT AAG TAT ATT CCT     2909
Gly Asn Thr Asp Ile Tyr Ser Ile Ile Phe Thr Asn Lys Tyr Ile Pro
    395                 400                 405

ATC CCA TAT ATA ACG TGT AAA AAG CTA AAG AAA TAC GAA TCC TTT GTT     2957
Ile Pro Tyr Ile Thr Cys Lys Lys Leu Lys Lys Tyr Glu Ser Phe Val
410                 415                 420                 425

GTA TAT GGA ACC GAA ATA AAA TCA ATA ATA AAA TCT TCA AAG ATT AGA     3005
Val Tyr Gly Thr Glu Ile Lys Ser Ile Ile Lys Ser Ser Lys Ile Arg
                430                 435                 440

TAT GCG AGT GTT ATA AAA GTA ACG GAG TAT ATC ACA TCT ATC TGT TCG     3053
Tyr Ala Ser Val Ile Lys Val Thr Glu Tyr Ile Thr Ser Ile Cys Ser
            445                 450                 455

GAA GAA ACT AGT TTA TGG AAC AGC ATC CCA ATT GAG ATA AAA CAT AAG     3101
Glu Glu Thr Ser Leu Trp Asn Ser Ile Pro Ile Glu Ile Lys His Lys
        460                 465                 470

ATT ATT AAT AAT ATA AAC AAT CAT GAT ATG TAT ATA TTA TAT AAA AAT     3149
Ile Ile Asn Asn Ile Asn Asn His Asp Met Tyr Ile Leu Tyr Lys Asn
    475                 480                 485

AGA AAA AAA AAA TAA                                                 3164
Arg Lys Lys Lys
490
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Lys Leu Leu Asn Tyr Asp Asn Phe Leu Arg Leu Lys Asn Leu Val Met
  1               5                  10                  15

Tyr Gly Ser His Ile Glu Asn Ile Ile Lys Asn Thr Tyr Met Tyr Tyr
             20                  25                  30

Ser Asn Ile Asp Lys Ala Ile Tyr Val Ile Met Lys His Cys Lys Lys
         35                  40                  45

His Ser Tyr Trp Met Arg Ile Pro Ile Glu Ile Gln Arg Tyr Ile Leu
     50                  55                  60

Leu His Leu Thr Met Lys Asp Leu Ser Ile Ile Leu Lys
 65                  70                  75
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 430 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Asp Asn Ile Arg Arg Ile Ile Ser Asn Ile Lys Gln Asp Asp Asn
  1               5                  10                  15

Ile Ala Thr Asp Met Leu Ala Thr Phe Leu Ser Ser Ser Leu His Val
             20                  25                  30

Phe Lys Leu Lys Glu Leu Lys Glu Ile Val Leu Leu Leu Leu Asn Lys
         35                  40                  45

Gly Ala Asn Leu Asn Gly Ile Ser Ile Tyr Asp Lys Thr Pro Phe His
     50                  55                  60

Cys Tyr Phe Thr Phe Asn Thr Asn Val Thr Ile Lys Val Ile Lys Phe
 65                  70                  75                  80

Leu Ile Tyr His Gly Gly Asp Ile Asn Ser Val His Arg Cys Gly Asp
                 85                  90                  95

Thr Ile Leu His Lys Tyr Leu Gly Asn Glu Asn Ile Asp Tyr Lys Val
            100                 105                 110

Val Glu Phe Leu Ile Arg Lys Gly Phe Asp Val Cys Lys Leu Asn Asn
        115                 120                 125

Ser Leu Lys Asn Pro Ile His Ile Phe Thr Ile Arg His Ile Asn Asn
    130                 135                 140

Thr Asn Leu Asn Ile Leu Asn Leu Leu Cys Ser His Ile Lys His Glu
```

145 150 155 160

Tyr Asn Lys Asn Asp Glu Met Met Ser Ile Leu Asn Thr Met Leu Asn
165 170 175

Tyr Cys His Asp Asp Tyr Thr Cys Phe Ser Ala Val Pro Tyr Thr Ile
180 185 190

Asp Ile Thr Thr Ile Asn Tyr Arg Asp Lys Leu Gly Tyr Ser Pro Val
195 200 205

Val Tyr Ala Ser Thr Thr Asp Lys Thr Ile Leu Val Asp Tyr Leu Ile
210 215 220

Lys Leu Gly Ala Asn Met Asn Ile Thr Thr Asn Asp Gly Asn Thr Cys
225 230 235 240

Gly Ser Phe Ala Val Met Asn Cys Asn Arg Asp Ile Asn Arg Leu Phe
245 250 255

Leu Asn Gln Asn Pro Asn Ile Glu Thr Ile Tyr Asn Thr Leu Lys Ile
260 265 270

Leu Ser Glu Asn Ile Val Phe Ile Asp Gly Cys Asp Val Arg Thr Asn
275 280 285

Met Val Lys Lys Ile Leu Met Tyr Gly Phe Thr Leu Asp Pro Leu Phe
290 295 300

Tyr Lys Asn His Asp Ile Ile Val Glu Tyr Phe Ser Ser Ser Ile Lys
305 310 315 320

Lys Tyr Asn Lys Ile Ile Leu Gln Met Ile Asp Glu Lys Ile Gly Asn
325 330 335

Arg Ser Val Tyr Asp Ile Ile Phe Thr Lys Ser Asn Thr Gly Met Asp
340 345 350

Val Arg Tyr Val Cys Asn Asp Ile Ile Ile Lys Tyr Ala Ser Val Lys
355 360 365

Tyr Tyr Gly Ser Leu Ile Lys Arg Leu Ile Tyr His Ser Lys Lys Arg
370 375 380

Lys Arg Asn Ile Leu Lys Ala Ile His Ala Met Glu Asn Asn Thr Thr
385 390 395 400

Leu Trp Asn Tyr Leu Pro Leu Glu Val Lys Met Tyr Ile Met Asp Phe
405 410 415

Leu Pro Asp Thr Asp Ile Thr Asn Ile Leu Phe Met Lys Lys
420 425 430

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 493 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Asp Asn Leu Tyr Arg Tyr Ile Thr Val Ser Asp Thr Val Asp Val
1 5 10 15

Asp Asn Val Arg Lys Leu Leu Ser Ser Cys Asn Ile Asp Val Val Ser
20 25 30

Thr Ile Phe Gln Lys Tyr Leu His Arg Asn Asp Ile Lys Leu Asp Ile
35 40 45

Val Glu Glu Phe Val Asn Asn Gly Ala Lys Leu Asn Gly Lys Asp Phe
50 55 60

Asn Asp Lys Asn Ile Pro Leu Cys Thr Leu Leu Ser Asn Lys Phe Ile
65 70 75 80

Asp Tyr Asn Ser Ala Ile Asp Ile Thr Ser Phe Met Ile Thr His Gly
85 90 95

Ala Asp Ile Asn Lys Arg Asn Lys Asp Gly Arg Thr Pro Ile Phe Cys
100 105 110

Leu Leu His Asn Ser Thr Leu Asn Asn Leu Glu Phe Val Ser Phe Met
115 120 125

Ile Asp His Gly Ala Asp Ile Thr Ile Val Asp Gly Phe Gly Phe Thr
130 135 140

Ser Leu Gln Ile Tyr Leu Gln Ser Ser Asn Val Gln Leu Asp Leu Val
145 150 155 160

Glu Leu Leu Ile Gln Lys Gly Val Asp Val Asn Ile His Asn Asn Trp
165 170 175

Phe Tyr Tyr Asn Thr Leu His Cys Tyr Ile Lys Lys Asn Tyr Asn Arg
180 185 190

Ile Asn Met Asp Ile Ile Lys Tyr Ile Met Asp Asn Gly Phe Thr Ile
195 200 205

Asn Glu Asn Lys Phe Thr Lys Ser Thr Phe Leu Asp Ile Leu Val Ser
210 215 220

Ile Ile Asp Ser Lys Asn Phe Asp Ser Asn Val Val Asp Phe Ile Leu
225 230 235 240

Lys Tyr Ile Asp Ile Asn Glu Lys Asn Ile Phe Asp Phe Thr Pro Leu
245 250 255

Tyr Cys Ser Val Asp Ala Asn Asn Glu Lys Met Cys Ser Tyr Leu Leu
260 265 270

Lys Lys Asn Ala Asp Pro Asn Ile Ile Thr Val Phe Gly Glu Thr Cys
275 280 285

Ile Leu Thr Ala Ile Asn Asn His Asn Lys Asn Ile Leu Tyr Lys Leu
290 295 300

Leu Asn Tyr Asp Ile Asp Ile Asn Thr Ile Gln Asn Thr Leu Phe Lys
305 310 315 320

Leu Glu Gln Asp Ile Ile Asn Ser Thr Ile Asp Thr Tyr Tyr Tyr Asn
325 330 335

Asn Leu Val Lys Lys Glu His Phe Ile Lys Leu Phe Leu Ala Tyr Ile
340 345 350

Val Lys Arg Tyr Glu Lys Asn Ile Gly Ile Leu Phe Leu Asp Tyr Pro
355 360 365

Thr Leu Gly Glu Tyr Phe Val Lys Phe Ile Asp Thr Cys Met Met Glu

```
            370                           375                           380

Ile Phe Glu Met Lys Ser Asp Lys Ala Gly Asn Thr Asp Ile Tyr Ser
385                 390                 395                 400

Ile Ile Phe Thr Asn Lys Tyr Ile Pro Ile Pro Tyr Ile Thr Cys Lys
                405                 410                 415

Lys Leu Lys Lys Tyr Glu Ser Phe Val Val Tyr Gly Thr Glu Ile Lys
            420                 425                 430

Ser Ile Ile Lys Ser Ser Lys Ile Arg Tyr Ala Ser Val Ile Lys Val
        435                 440                 445

Thr Glu Tyr Ile Thr Ser Ile Cys Ser Glu Glu Thr Ser Leu Trp Asn
    450                 455                 460

Ser Ile Pro Ile Glu Ile Lys His Lys Ile Ile Asn Asn Ile Asn Asn
465                 470                 475                 480

His Asp Met Tyr Ile Leu Tyr Lys Asn Arg Lys Lys Lys
                485                 490
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3295 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Porcine Reproductive and Respiratory Virus
(B) STRAIN: IA-2 Strain (NVSL)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 47..814

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 670..1431

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1215..1748

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1762..2361

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2349..2870

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2863..3231

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GAATTCCCGG GCCCTGTCAT TGAACCAACT TTAGGCCTGA ATTGAAATGA AATGGGGTCC 60
ATGCAAAGCC TTTTTGACAA AATTGGCCAA CTTTTTGTGG ATGCTTTCAC GGAGTTCTTG 120
GTGTCCATTG TTGATATCAT TATATTTTTG GCCATTTTGT TTGGCTTCAC CATCGCCGGT 180
TGGTTGGTGG TCTTTTGCAT CAGATTGGTT TGCTCCGCGA TACTCCGTAC GCGCCCTGCC 240
ATTCACTCTG AGCAATTACA GAAGATCTTA TGAAGCCTTT CTTTCCCAGT GCCAAGTGGA 300
CATTCCCACC TGGGGAACTA AACATCCTTT GGGGATGTTT TGGCACCATA AGGTGTCAAC 360
CCTGATTGAT GAGATGGTGT CGCGTCGAAT GTACCGCATC ATGGAAAAAG CAGGACAGGC 420
TGCCTGGAAA CAGGTGGTGA GCGAGGCTAC GCTGTCTCGC ATTAGTAGTT TGGATGTGGT 480
GGCTCATTTT CAGCATCTTG CCGCCATTGA AGCCGAGACC TGTAAATATT TGGCCTCCCG 540
GCTGCCCATG CTACACAACC TGCGCATGAC AGGGTCAAAT GTAACCATAG TGTATAATAG 600
TACTTTGCAT CAGGTGTTTG CTATTTTTCC AACCCCTGGT TCCCGGCCAA AGCTTCATGA 660
TTTTCAGCAA TGGTTAATAG CTGTACATTC CTCCATATTT TCCTCTGTTG CAGCTTCTTG 720
TACTCTCTTT GTTGTGCTGT GGTTGCGGGT TCCAATACTA CGTACTGTTT TTGGTTTCCG 780
CTGGTTAGGG GCAATTTTTC TTTCGAACTC ACAGTGAATT ACACGGTGTG TCCACCTTGC 840
CTCACCCGGC AAGCAGCCGC AGAGGCCTAC GAACCCGGTA GGTCTCTTTG GTGCAGGATA 900
GGGTATGACC GATGTGGGGA GGACGATCAT GACGAGCTAG GGTTTATGGT ACCGTCTGGC 960
CTCTCCAGCG AAGGCCACTT GACCAGTGTT TACGCCTGGT TGGCGTTCTT GTCCTTCAGC 1020
TACACGGCCC AGTTCCATCC CGAGATATTC GGGATAGGGA ATGTGAGTCG AGTTTATGTT 1080
GACATCGAAC ATCAACTCAT CTGCGCCGAA CATGACGGGC AGAACACCAC CTTGCCTCGT 1140
CATGACAACA TTTCAGCCGT GTTTCAGACC TATTACCAAC ATCAAGTCGA CGGCGGCAAT 1200
TGGTTTCACC TAGAATGGCT GCGTCCCTTC TTTTCCTCAT GGTTGGTTTT AAATGTCTCT 1260
TGGTTTCTCA GGCGTTCGCC TGCAAACCAT GTTTCAGTTC GAGTCTTGCA GACATTAAGA 1320
CCAACACCAC CGCAGCGGCA AGCTTTGCTG TCCTCCAAGA CATCAGTTGC CTTAGGCATC 1380
GCAACTCGGC CTCTGAGGCG ATTCGCAAAA TCCCTCAGTG CCGTACGGCG ATAGGGACAC 1440
CCGTGTATAT TACCACCACA GCCAATGTGA CAGATGAGAA TTATTTACAT TCTTCTGATC 1500
TCCTCATGCT TTCTTCTTGC CTTTTCTATG CTTCTGAGAT GAGTGAAAAG GGATTTAAGG 1560
TGGTATTTGG CAATGTGTCA GGCATCGTGG CTGTGTGTGT CAATTTTACC AGCTACGTCC 1620
AACATGTCAG GGAGTTTACC CAACGCTCCT TGATGGTCGA CCATGTGCGG CTGCTCCATT 1680
TCATGACACC TGAGACCATG AGGTGGGCAA CTGTTTTAGC CTGTCTTTTT GCCATTCTGT 1740
TGGCAATTTG AATGTTTAAG TATGTTGGGG AAATGCTTGA CCGCGGGCTG TTGCTCGCGA 1800
TTGCTTTCTT TGTGGTGTAT CGTGCCGTTC TGTTTTGCTG TGCTCGTCAA CGCCAACAGC 1860
AACAGCAGCT CTCATCTACA GTTGATTTAC AACTTGACGC TATGTGAGCT GAATGGCACA 1920
GATTGGCTAT CTAATAAATT TGATTGGGCA GTGGAGAGTT TTGTCATCTT TCCCGTTTTG 1980
ACTCACATTG TCTCCTATGG TGCCCTCACT ACCAGCCATT TCCTTGACAC AGTCGCTTTA 2040

MISSING UPON TIME OF PUBLICATION

Pro Phe Thr Leu Ser Asn Tyr Arg Arg Ser Tyr Glu Ala Phe Leu Ser
65 70 75 80

Gln Cys Gln Val Asp Ile Pro Thr Trp Gly Thr Lys His Pro Leu Gly
85 90 95

Met Phe Trp His His Lys Val Ser Thr Leu Ile Asp Glu Met Val Ser
100 105 110

Arg Arg Met Tyr Arg Ile Met Glu Lys Ala Gly Gln Ala Ala Trp Lys
115 120 125

Gln Val Val Ser Glu Ala Thr Leu Ser Arg Ile Ser Ser Leu Asp Val
130 135 140

Val Ala His Phe Gln His Leu Ala Ala Ile Glu Ala Glu Thr Cys Lys
145 150 155 160

Tyr Leu Ala Ser Arg Leu Pro Met Leu His Asn Leu Arg Met Thr Gly
165 170 175

Ser Asn Val Thr Ile Val Tyr Asn Ser Thr Leu His Gln Val Phe Ala
180 185 190

Ile Phe Pro Thr Pro Gly Ser Arg Pro Lys Leu His Asp Phe Gln Gln
195 200 205

Trp Leu Ile Ala Val His Ser Ser Ile Phe Ser Ser Val Ala Ala Ser
210 215 220

Cys Thr Leu Phe Val Val Leu Trp Leu Arg Val Pro Ile Leu Arg Thr
225 230 235 240

Val Phe Gly Phe Arg Trp Leu Gly Ala Ile Phe Leu Ser Asn Ser Gln
245 250 255

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 254 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Val Asn Ser Cys Thr Phe Leu His Ile Phe Leu Cys Cys Ser Phe
1 5 10 15

Leu Tyr Ser Leu Cys Cys Ala Val Val Ala Gly Ser Asn Thr Thr Tyr
20 25 30

Cys Phe Trp Phe Pro Leu Val Arg Gly Asn Phe Ser Phe Glu Leu Thr
35 40 45

Val Asn Tyr Thr Val Cys Pro Pro Cys Leu Thr Arg Gln Ala Ala Ala
50 55 60

Glu Ala Tyr Glu Pro Gly Arg Ser Leu Trp Cys Arg Ile Gly Tyr Asp
65 70 75 80

Arg Cys Gly Glu Asp Asp His Asp Glu Leu Gly Phe Met Val Pro Ser
85 90 95

Gly Leu Ser Ser Glu Gly His Leu Thr Ser Val Tyr Ala Trp Leu Ala

100 105 110

Phe Leu Ser Phe Ser Tyr Thr Ala Gln Phe His Pro Glu Ile Phe Gly
115 120 125

Ile Gly Asn Val Ser Arg Val Tyr Val Asp Ile Glu His Gln Leu Ile
130 135 140

Cys Ala Glu His Asp Gly Gln Asn Thr Thr Leu Pro Arg His Asp Asn
145 150 155 160

Ile Ser Ala Val Phe Gln Thr Tyr Tyr Gln His Gln Val Asp Gly Gly
165 170 175

Asn Trp Phe His Leu Glu Trp Leu Arg Pro Phe Phe Ser Ser Trp Leu
180 185 190

Val Leu Asn Val Ser Trp Phe Leu Arg Arg Ser Pro Ala Asn His Val
195 200 205

Ser Val Arg Val Leu Gln Thr Leu Arg Pro Thr Pro Pro Gln Arg Gln
210 215 220

Ala Leu Leu Ser Ser Lys Thr Ser Val Ala Leu Gly Ile Ala Thr Arg
225 230 235 240

Pro Leu Arg Arg Phe Ala Lys Ser Leu Ser Ala Val Arg Arg
245 250

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 178 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Ala Ala Ser Leu Leu Phe Leu Met Val Gly Phe Lys Cys Leu Leu
1 5 10 15

Val Ser Gln Ala Phe Ala Cys Lys Pro Cys Phe Ser Ser Ser Leu Ala
20 25 30

Asp Ile Lys Thr Asn Thr Thr Ala Ala Ala Ser Phe Ala Val Leu Gln
35 40 45

Asp Ile Ser Cys Leu Arg His Arg Asn Ser Ala Ser Glu Ala Ile Arg
50 55 60

Lys Ile Pro Gln Cys Arg Thr Ala Ile Gly Thr Pro Val Tyr Ile Thr
65 70 75 80

Thr Thr Ala Asn Val Thr Asp Glu Asn Tyr Leu His Ser Ser Asp Leu
85 90 95

Leu Met Leu Ser Ser Cys Leu Phe Tyr Ala Ser Glu Met Ser Glu Lys
100 105 110

Gly Phe Lys Val Val Phe Gly Asn Val Ser Gly Ile Val Ala Val Cys
115 120 125

Val Asn Phe Thr Ser Tyr Val Gln His Val Arg Glu Phe Thr Gln Arg
130 135 140

Ser Leu Met Val Asp His Val Arg Leu Leu His Phe Met Thr Pro Glu
145 150 155 160

Thr Met Arg Trp Ala Thr Val Leu Ala Cys Leu Phe Ala Ile Leu Leu
165 170 175

Ala Ile

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 200 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met Leu Gly Lys Cys Leu Thr Ala Gly Cys Cys Ser Arg Leu Leu Ser
1 5 10 15

Leu Trp Cys Ile Val Pro Phe Cys Phe Ala Val Leu Val Asn Ala Asn
20 25 30

Ser Asn Ser Ser Ser His Leu Gln Leu Ile Tyr Asn Leu Thr Leu Cys
35 40 45

Glu Leu Asn Gly Thr Asp Trp Leu Ser Asn Lys Phe Asp Trp Ala Val
50 55 60

Glu Ser Phe Val Ile Phe Pro Val Leu Thr His Ile Val Ser Tyr Gly
65 70 75 80

Ala Leu Thr Thr Ser His Phe Leu Asp Thr Val Ala Leu Val Thr Val
85 90 95

Ser Thr Ala Gly Phe Val His Gly Arg Tyr Val Leu Ser Ser Ile Tyr
100 105 110

Ala Val Cys Ala Leu Ala Ala Leu Thr Cys Phe Val Ile Arg Phe Ala
115 120 125

Lys Asn Cys Met Ser Trp Arg Tyr Ser Cys Thr Arg Tyr Thr Asn Phe
130 135 140

Leu Leu Asp Thr Lys Gly Arg Leu Tyr Arg Trp Arg Ser Pro Val Ile
145 150 155 160

Ile Glu Lys Arg Gly Lys Val Glu Val Glu Gly His Leu Ile Asp Leu
165 170 175

Lys Arg Val Val Leu Asp Gly Ser Val Ala Thr Pro Ile Thr Arg Val
180 185 190

Ser Ala Glu Gln Trp Gly Arg Pro
195 200

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 174 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Met Gly Ser Ser Leu Asp Asp Phe Cys Tyr Asp Ser Thr Ala Pro Gln
 1               5                  10                  15

Lys Val Leu Leu Ala Phe Ser Ile Thr Tyr Thr Pro Val Met Ile Tyr
            20                  25                  30

Ala Leu Lys Val Ser Arg Gly Arg Leu Leu Gly Leu Leu His Leu Leu
        35                  40                  45

Ile Phe Leu Asn Cys Ala Phe Thr Phe Gly Tyr Met Thr Phe Ala His
    50                  55                  60

Phe Gln Ser Thr Asn Lys Val Ala Leu Thr Met Gly Ala Val Val Ala
65                  70                  75                  80

Leu Leu Trp Gly Val Tyr Ser Ala Ile Glu Thr Trp Lys Phe Ile Thr
                85                  90                  95

Ser Arg Cys Arg Leu Cys Leu Leu Gly Arg Lys Tyr Ile Leu Ala Pro
            100                 105                 110

Ala His His Val Glu Ser Ala Ala Gly Phe His Pro Ile Ala Ala Asn
        115                 120                 125

Asp Asn His Ala Phe Val Val Arg Arg Pro Gly Ser Thr Thr Val Asn
    130                 135                 140

Gly Thr Leu Val Pro Gly Leu Lys Gly Leu Val Leu Gly Gly Arg Lys
145                 150                 155                 160

Ala Val Lys Gln Gly Val Val Asn Leu Val Lys Tyr Ala Lys
                165                 170
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 123 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
Met Pro Asn Asn Asn Gly Lys Gln Gln Lys Arg Lys Lys Gly Asp Gly
 1               5                  10                  15

Gln Pro Val Asn Gln Leu Cys Gln Met Leu Gly Lys Ile Ile Ala Gln
            20                  25                  30

Gln Asn Gln Ser Arg Gly Lys Gly Pro Gly Lys Lys Asn Lys Lys Lys
        35                  40                  45

Asn Pro Glu Lys Pro His Phe Pro Leu Ala Thr Glu Asp Asp Val Arg
    50                  55                  60

His His Phe Thr Pro Ser Glu Arg Gln Leu Cys Leu Ser Ser Ile Gln
65                  70                  75                  80

Thr Ala Phe Asn Gln Gly Ala Gly Thr Cys Thr Leu Ser Asp Ser Gly
                85                  90                  95

Arg Ile Ser Tyr Thr Val Glu Phe Ser Leu Pro Thr His His Thr Val
```

100 105 110

Arg Leu Ile Arg Val Thr Ala Ser Pro Ser Ala
115 120

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GCGGATCCGG CGCGCCGGAT TTTCCTACAT CTACACT 37

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CTAAAATTGA ATTGTAAT 18

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TTGGCGCGCC CTAGATCTGT GTAGTTGATT GATTTG 36

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TACGGCGCGC CGGGAAATGC TAAAGCCAAG CCCACA 36

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TTCGGATCCT GCTCAGACAG TATTGTGTAT GTTATCAAGA GC 42

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CCATGAATTC CTTCCCTGAA TGCAAGGAGG GCTTC 35

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CGGGATCCTC ACCCGGGCAG CGCGCTGTA 29

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CGGAATTCAC AAGGGCCGAC ATTGGCC 27

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

ATCGGGATCC CGTTATTCTT CGCTGATGGT GG 32

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

ATCGGAATTC GCGGTGCCTG TTGCTCTGGA TG 32

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CTTCGGATCC TCATGCCCCC CCGACGTCGG CCATC 35

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

TCATGAATTC GGCCGCTCGC GGCGGTGCTG AACGC 35

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CGGGATCCCT AGGGCGCGGA GCCGAGGGC 29

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CGGAATTCAG GCCCGCTGGG GCGAGCGTGG 30

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CTTCGGATCC TCATGCCCCC CCGACGTCGG CCATC 35

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

TCATGAATTC GGCCGCTCGC GGCGGTGCTG AACGC 35

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CGGGATCCTT AATATAATTT TCTAGGTGCT AGTTG 35

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CGGAATTCGA TGAGTGATGG AGCAGTTCAA 30

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

CGGGATCCTT AATATAATTT TCTAGGTGCT AGTTG 35

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

CGGAATTCGA TGAGTGATGG AGCAGTTCAA 30

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

CGGGATCCTT AATATAATTT TCTAGGTGCT AGTTG 35

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

CGGAATTCTA TGTGTTTTTT TATAGGACTT 30

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

CGGGATCCTT AATATAATTT TCTAGGTGCT AGTTG 35

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

CGGAATTCTA TGTGTTTTTT TATAGGACTT 30

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

CGTCAGATCT CAGGAGGTCA TAAGATGCCA TTAGC 35

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

CGTTGAATTC GATGACTTGC CAGACTTACA ACTTG 35

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

CGTCGAATTC GATGTCTGGA GCCTCTAGTG GGA 33

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

CGTCGGATCC GGCTCAAATA GCCGATACTC TTCTT 35

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

CGTCGAATTC AATGGAAAGT CCAACGCACC CAAAA 35

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

CGTCGGATCC GGGGACTAAA TGGAATCATA CA 32

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

CGGGAATTCG GGGTCGTCCT TAGATGACTT CTGCC 35

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

GCGGATCCTT GTTATGTGGC ATATTTGACA AGGTTTAC 38

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

AATGAATTCG AAATGGGGTC CATGCAAAGC CTTTTTG 37

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

CAAGGATCCC ACACCGTGTA ATTCACTGTG AGTTCG 36

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

GTCGAATTCG CCAAATAACA ACGGCAAGCA GCAGAAG 37

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

CAAGGATCCC AGCCCATCAT GCTGAGGGTG ATG 33

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

TTCGAATTCG GCTAATAGCT GTACATTCCT CCATATTT 38

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

GGGGATCCTA TCGCCGTACG GCACTGAGGG 30

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

CCGAATTCGG CTGCGTCCCT TCTTTTCCTC ATGG 34

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

CTGGATCCTT CAAATTGCCA ACAGAATGGC AAAAAGAC 38

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

TTGAATTCGT TGGAGAAATG CTTGACCGCG GGC 33

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

GAAGGATCCT AAGGACGACC CCATTGTTCC GCTG 34

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

ATGAAGGCCC TGTACCCCGT CACGA 25

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

CGGGATCCGG CTACAGGGCG TCGGGGTCCT C 31

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

CCGGATCCGG CGCGCGACGT GACCCGGCTC 30

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

MISSING UPON TIME OF PUBLICATION

225 230 235 240

Asn Met Phe Pro Ala Ile Ile Pro Ser Val Asn Asp Phe Ile Ser Thr
245 250 255
Val Val Asp Lys Asp Arg Leu Ile Asn Met Tyr Gly Ile Lys Cys Val
260 265 270
Ala Met Phe Ser Tyr Asp Ile Asn Met Ile Asp Leu Glu Ser Leu Asp
275 280 285
Asp Ser Asp Tyr Ile Phe Ile Glu Lys Asn Ile Ser Ile Tyr Asp Val
290 295 300
Lys Cys Arg Asp Phe Ala Asn Met Ile Arg Asp Lys Val Lys Arg Glu
305 310 315 320

Lys Asn Arg Ile Leu Thr Thr Lys Cys Glu Asp Ile Ile Arg Tyr Ile
325 330 335
Lys Leu Phe Ser Lys Asn Arg Ile Asn Asp Glu Asn Asn Lys Val Glu
340 345 350
Glu Val Leu Ile His Ile Asp Asn Val Ser Lys Asn Asn Lys Leu Ser
355 360 365
Leu Ser Asp Ile Ser Ser Leu Met Asp Gln Phe Arg Leu Asn Pro Cys
370 375 380

Thr Ile Arg Asn Ile Leu Leu Ser Ser Ala Thr Ile Lys Ser Lys Leu
385 390 395 400

Leu Ala Leu Arg Ala Val Lys Asn Trp Lys Cys Tyr Ser Leu Thr Asn
405 410 415

Val Ser Met Tyr Lys Lys Ile Lys Gly Val Ile Val Met Asp Met Val
420 425 430

Asp Tyr Ile Ser Thr Asn Ile Leu Lys Tyr His Lys Gln Leu Tyr Asp
435 440 445

Lys Met Ser Thr Phe Glu Tyr Lys Arg Asp Ile Lys Ser Cys Lys Cys
450 455 460

Ser Ile Cys Ser Asp Ser Ile Thr His His Ile Tyr Glu Thr Thr Ser
465 470 475 480

Cys Ile Asn Tyr Lys Ser Thr Asp Asn Asp Leu Met Ile Val Leu Phe
485 490 495
Asn Leu Thr Arg Tyr Leu Met His Gly Met Ile His Pro Asn Leu Ile
500 505 510
Ser Val Lys Gly Trp Gly Pro Leu Ile Gly Leu Leu Thr Gly Asp Ile
515 520 525
Gly Ile Asn Leu Lys Leu Tyr Ser Thr Met Asn Ile Asn Gly Leu Arg
530 535 540
Tyr Gly Asp Ile Thr Leu Ser Ser Tyr Asp Met Ser Asn Lys Leu Val
545 550 555 560

Ser Ile Ile Asn Thr Pro Ile Tyr Glu Leu Ile Pro Phe Thr Thr Cys
565 570 575
Cys Ser Leu Asn Glu Tyr Tyr Ser Lys Ile Val Ile Leu Ile Asn Val
580 585 590
Ile Leu Glu Tyr Met Ile Ser Ile Ile Leu Tyr Arg Ile Leu Ile Val
595 600 605

Lys Arg Phe Asn Asn Ile Lys Glu Phe Ile Ser Lys Val Val Asn Thr
610 615 620

Val Leu Glu Ser Ser Gly Ile Tyr Phe Cys Gln Met Arg Val His Glu
625 630 635 640

Gln Ile Glu Leu Glu Ile Asp Glu Leu Ile Ile Asn Gly Ser Met Pro

645 650 655

Val Gln Leu Met His Leu Leu Leu Lys Val Ala Thr Ile Ile Leu Glu
660 665 670

Glu Ile Lys Glu Ile
675

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

TCGAAGATCT TCTCATGCAA AGGTGGAACC GTTC 34

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

TCGAAGATCT CATGCCTATG TTCACCATCC ACAC 34

(2) INFORMATION FOR SEQ ID NO:189:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3942 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox virus
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: S-SPV-001

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..369

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 370..597

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 598..1539

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1675..3708

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: complement (3748..3942)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:189:

```
TGT TTG TTC ATT AAT AAG ATG GGT GGA GCT ATT ATA GAA TAC AAG ATA        48
Cys Leu Phe Ile Asn Lys Met Gly Gly Ala Ile Ile Glu Tyr Lys Ile
  1               5                  10                  15

CCT GGT TCC AAA TCT ATA ACC AAA TCT ATT TCC GAA GAA CTA GAA AAT        96
Pro Gly Ser Lys Ser Ile Thr Lys Ser Ile Ser Glu Glu Leu Glu Asn
             20                  25                  30

TTA ACA AAG CGA GAT AAA CCA ATA TCT AAA ATT ATA GTT ATT CCT ATT       144
Leu Thr Lys Arg Asp Lys Pro Ile Ser Lys Ile Ile Val Ile Pro Ile
         35                  40                  45

GTA TGT TAC AGA AAT GCA AAT AGT ATA AAG GTT ACA TTT GCA CTA AAA       192
Val Cys Tyr Arg Asn Ala Asn Ser Ile Lys Val Thr Phe Ala Leu Lys
     50                  55                  60

AAG TTT ATC ATA GAT AAG GAG TTT AGT ACA AAT GTA ATA GAC GTA GAT       240
Lys Phe Ile Ile Asp Lys Glu Phe Ser Thr Asn Val Ile Asp Val Asp
 65                  70                  75                  80

GGT AAA CAT GAA AAA ATG TCC ATG AAT GAA ACA TGC GAA GAG GAT GTT       288
Gly Lys His Glu Lys Met Ser Met Asn Glu Thr Cys Glu Glu Asp Val
                 85                  90                  95

GCT AGA GGA TTG GGA ATT ATA GAT CTT GAA GAT GAA TGC ATA GAG GAA       336
Ala Arg Gly Leu Gly Ile Ile Asp Leu Glu Asp Glu Cys Ile Glu Glu
            100                 105                 110

GAT GAT GTC GAT ACG TCA TTA TTT AAT GTA TAAATG GAT AAA TTG TAT        384
Asp Asp Val Asp Thr Ser Leu Phe Asn Val     Met Asp Lys Leu Tyr
        115                 120                  1               5

GCG GCA ATA TTC GGC GTT TTT ATG ACA TCT AAA GAT GAT GAT TTT AAT       432
Ala Ala Ile Phe Gly Val Phe Met Thr Ser Lys Asp Asp Asp Phe Asn
                 10                  15                  20

AAC TTT ATA GAA GTT GTA AAA TCT GTA TTA ACA GAT ACA TCA TCT AAT       480
```

```
Asn Phe Ile Glu Val Val Lys Ser Val Leu Thr Asp Thr Ser Ser Asn
            25                  30                  35

CAT ACA ATA TCG TCG TCC AAT AAT AAT ACA TGG ATA TAT ATA TTT CTA     528
His Thr Ile Ser Ser Ser Asn Asn Asn Thr Trp Ile Tyr Ile Phe Leu
        40                  45                  50

GCG ATA TTA TTT GGT GTT ATG GTA TTA TTA GTT TTT ATT TTG TAT TTA     576
Ala Ile Leu Phe Gly Val Met Val Leu Leu Val Phe Ile Leu Tyr Leu
    55                  60                  65

AAA GTT ACT AAA CCA ACT TAAATG GAG GAA GCA GAT AAC CAA CTC GTT     624
Lys Val Thr Lys Pro Thr     Met Glu Glu Ala Asp Asn Gln Leu Val
70                  75        1               5

TTA AAT AGT ATT AGT GCT AGA GCA TTA AAG GCA TTT TTT GTA TCT AAA     672
Leu Asn Ser Ile Ser Ala Arg Ala Leu Lys Ala Phe Phe Val Ser Lys
10                  15                  20                  25

ATT AAT GAT ATG GTC GAT GAA TTA GTT ACC AAA AAA TAT CCA CCA AAG     720
Ile Asn Asp Met Val Asp Glu Leu Val Thr Lys Lys Tyr Pro Pro Lys
                30                  35                  40

AAG AAA TCA CAA ATA AAA CTC ATA GAT ACA CGA ATT CCT ATT GAT CTT     768
Lys Lys Ser Gln Ile Lys Leu Ile Asp Thr Arg Ile Pro Ile Asp Leu
            45                  50                  55

ATT AAT CAA CAA TTC GTT AAA AGA TTT AAA CTA GAA AAT TAT AAA AAT     816
Ile Asn Gln Gln Phe Val Lys Arg Phe Lys Leu Glu Asn Tyr Lys Asn
        60                  65                  70

GGA ATT TTA TCC GTT CTT ATC AAT AGT TTA GTC GAA AAT AAT TAC TTT     864
Gly Ile Leu Ser Val Leu Ile Asn Ser Leu Val Glu Asn Asn Tyr Phe
    75                  80                  85

GAA CAA GAT GGT AAA CTT AAT AGC AGT GAT ATT GAT GAA TTA GTG CTC     912
Glu Gln Asp Gly Lys Leu Asn Ser Ser Asp Ile Asp Glu Leu Val Leu
90                  95                  100                 105

ACA GAC ATA GAG AAA AAG ATT TTA TCG TTG ATT CCT AGA TGT TCT CCT     960
Thr Asp Ile Glu Lys Lys Ile Leu Ser Leu Ile Pro Arg Cys Ser Pro
                110                 115                 120

CTT TAT ATA GAT ATC AGT GAC GTT AAA GTT CTC GCA TCT AGG TTA AAA    1008
Leu Tyr Ile Asp Ile Ser Asp Val Lys Val Leu Ala Ser Arg Leu Lys
            125                 130                 135

AAA AGT GCT AAA TCA TTT ACG TTT AAT GAT CAT GAA TAT ATT ATA CAA    1056
Lys Ser Ala Lys Ser Phe Thr Phe Asn Asp His Glu Tyr Ile Ile Gln
        140                 145                 150

TCT GAT AAA ATA GAG GAA TTA ATA AAT AGT TTA TCT AGA AAC CAT GAT    1104
Ser Asp Lys Ile Glu Glu Leu Ile Asn Ser Leu Ser Arg Asn His Asp
    155                 160                 165

ATT ATA CTA GAT GAA AAA AGT TCT ATT AAA GAC AGC ATA TAT ATA CTA    1152
Ile Ile Leu Asp Glu Lys Ser Ser Ile Lys Asp Ser Ile Tyr Ile Leu
170                 175                 180                 185

TCT GAT GAT CTT TTG AAT ATA CTT CGT GAA AGA TTA TTT AGA TGT CCA    1200
Ser Asp Asp Leu Leu Asn Ile Leu Arg Glu Arg Leu Phe Arg Cys Pro
                190                 195                 200

CAG GTT AAA GAT AAT ACT ATT TCT AGA ACA CGT CTA TAT GAT TAT TTT    1248
Gln Val Lys Asp Asn Thr Ile Ser Arg Thr Arg Leu Tyr Asp Tyr Phe
            205                 210                 215
```

```
ACT AGA GTG TCA AAG AAA GAA GAA GCG AAA ATA TAC GTT ATA TTG AAA    1296
Thr Arg Val Ser Lys Lys Glu Glu Ala Lys Ile Tyr Val Ile Leu Lys
        220                 225                 230

GAT TTA AAG ATT GCT GAT ATA CTC GGT ATC GAA ACA GTA ACG ATA GGA    1344
Asp Leu Lys Ile Ala Asp Ile Leu Gly Ile Glu Thr Val Thr Ile Gly
    235                 240                 245

TCA TTT GTA TAT ACG AAA TAT AGC ATG TTG ATT AAT TCA ATT TCG TCT    1392
Ser Phe Val Tyr Thr Lys Tyr Ser Met Leu Ile Asn Ser Ile Ser Ser
250                 255                 260                 265

AAT GTT GAT AGA TAT TCA AAA AGG TTC CAT GAC TCT TTT TAT GAA GAT    1440
Asn Val Asp Arg Tyr Ser Lys Arg Phe His Asp Ser Phe Tyr Glu Asp
                270                 275                 280

ATT GCG GAA TTT ATA AAG GAT AAT GAA AAA ATT AAT GTA TCC AGA GTT    1488
Ile Ala Glu Phe Ile Lys Asp Asn Glu Lys Ile Asn Val Ser Arg Val
            285                 290                 295

GTT GAA TGC CTT ATC GTA CCT AAT ATT AAT ATA GAG TTA TTA ACT GAA    1536
Val Glu Cys Leu Ile Val Pro Asn Ile Asn Ile Glu Leu Leu Thr Glu
        300                 305                 310

TAAGTATATA TAAATGATTG TTTTTATAAT GTTTGTTATC GCATTTAGTT TTGCTGTATG  1596

GTTATCATAT ACATTTTTAA GGCCGTATAT GATAAATGAA AATATATAAG CACTTATTTT  1656

TGTTAGTATA ATAACACA ATG CCG TCG TAT ATG TAT CCG AAG AAC GCA AGA    1707
                    Met Pro Ser Tyr Met Tyr Pro Lys Asn Ala Arg
                      1               5                  10

AAA GTA ATT TCA AAG ATT ATA TCA TTA CAA CTT GAT ATT AAA AAA CTT    1755
Lys Val Ile Ser Lys Ile Ile Ser Leu Gln Leu Asp Ile Lys Lys Leu
            15                  20                  25

CCT AAA AAA TAT ATA AAT ACC ATG TTA GAA TTT GGT CTA CAT GGA AAT    1803
Pro Lys Lys Tyr Ile Asn Thr Met Leu Glu Phe Gly Leu His Gly Asn
        30                  35                  40

CTA CCA GCT TGT ATG TAT AAA GAT GCC GTA TCA TAT GAT ATA AAT AAT    1851
Leu Pro Ala Cys Met Tyr Lys Asp Ala Val Ser Tyr Asp Ile Asn Asn
    45                  50                  55

ATA AGA TTT TTA CCT TAT AAT TGT GTT ATG GTT AAA GAT TTA ATA AAT    1899
Ile Arg Phe Leu Pro Tyr Asn Cys Val Met Val Lys Asp Leu Ile Asn
60                  65                  70                  75

GTT ATA AAA TCA TCA TCT GTA ATA GAT ACT AGA TTA CAT CAA TCT GTA    1947
Val Ile Lys Ser Ser Ser Val Ile Asp Thr Arg Leu His Gln Ser Val
                80                  85                  90

TTA AAA CAT CGT AGA GCG TTA ATA GAT TAC GGC GAT CAA GAC ATT ATC    1995
Leu Lys His Arg Arg Ala Leu Ile Asp Tyr Gly Asp Gln Asp Ile Ile
            95                  100                 105

ACT TTA ATG ATC ATT AAT AAG TTA CTA TCG ATA GAT GAT ATA TCC TAT    2043
Thr Leu Met Ile Ile Asn Lys Leu Leu Ser Ile Asp Asp Ile Ser Tyr
        110                 115                 120

ATA TTA GAT AAA AAA ATA ATT CAT GTA ACA AAA ATA TTA AAA ATA GAC    2091
Ile Leu Asp Lys Lys Ile Ile His Val Thr Lys Ile Leu Lys Ile Asp
    125                 130                 135

CCT ACA GTA GCC AAT TCA AAC ATG AAA CTG AAT AAG ATA GAG CTT GTA    2139
Pro Thr Val Ala Asn Ser Asn Met Lys Leu Asn Lys Ile Glu Leu Val
140                 145                 150                 155
```

```
GAT GTA ATA ACA TCA ATA CCT AAG TCT TCC TAT ACA TAT TTA TAT AAT    2187
Asp Val Ile Thr Ser Ile Pro Lys Ser Ser Tyr Thr Tyr Leu Tyr Asn
                160                 165                 170

AAT ATG ATC ATT GAT CTC GAT ACA TTA TTA TAT TTA TCC GAT GCA TTC    2235
Asn Met Ile Ile Asp Leu Asp Thr Leu Leu Tyr Leu Ser Asp Ala Phe
            175                 180                 185

CAC ATA CCC CCC ACA CAT ATA TCA TTA CGT TCA CTT AGA GAT ATA AAC    2283
His Ile Pro Pro Thr His Ile Ser Leu Arg Ser Leu Arg Asp Ile Asn
        190                 195                 200

AGG ATT ATT GAA TTG CTT AAA AAA TAT CCG AAT AAT AAT ATT ATT GAT    2331
Arg Ile Ile Glu Leu Leu Lys Lys Tyr Pro Asn Asn Asn Ile Ile Asp
    205                 210                 215

TAT ATA TCC GAT AGC ATA AAA TCA AAT AGT TCA TTC ATT CAC ATA CTT    2379
Tyr Ile Ser Asp Ser Ile Lys Ser Asn Ser Ser Phe Ile His Ile Leu
220                 225                 230                 235

CAT ATG ATA ATA TCA AAT ATG TTT CCT GCT ATA ATC CCT AGT GTA AAC    2427
His Met Ile Ile Ser Asn Met Phe Pro Ala Ile Ile Pro Ser Val Asn
                240                 245                 250

GAT TTT ATA TCT ACC GTA GTT GAT AAA GAT CGA CTT ATT AAT ATG TAT    2475
Asp Phe Ile Ser Thr Val Val Asp Lys Asp Arg Leu Ile Asn Met Tyr
            255                 260                 265

GGG ATT AAG TGT GTT GCT ATG TTT TCG TAC GAT ATA AAC ATG ATC GAT    2523
Gly Ile Lys Cys Val Ala Met Phe Ser Tyr Asp Ile Asn Met Ile Asp
        270                 275                 280

TTA GAG TCA TTA GAT GAC TCA GAT TAC ATA TTT ATA GAA AAA AAT ATA    2571
Leu Glu Ser Leu Asp Asp Ser Asp Tyr Ile Phe Ile Glu Lys Asn Ile
    285                 290                 295

TCT ATA TAC GAC GTT AAA TGT AGA GAT TTT GCG AAT ATG ATT AGA GAT    2619
Ser Ile Tyr Asp Val Lys Cys Arg Asp Phe Ala Asn Met Ile Arg Asp
300                 305                 310                 315

AAG GTT AAA AGA GAA AAG AAT AGA ATA TTA ACT ACG AAA TGT GAA GAT    2667
Lys Val Lys Arg Glu Lys Asn Arg Ile Leu Thr Thr Lys Cys Glu Asp
                320                 325                 330

ATT ATA AGA TAT ATA AAA TTA TTC AGT AAA AAT AGA ATA AAC GAT GAA    2715
Ile Ile Arg Tyr Ile Lys Leu Phe Ser Lys Asn Arg Ile Asn Asp Glu
            335                 340                 345

AAT AAT AAG GTG GAG GAG GTG TTG ATA CAT ATT GAT AAT GTA TCT AAA    2763
Asn Asn Lys Val Glu Glu Val Leu Ile His Ile Asp Asn Val Ser Lys
        350                 355                 360

AAT AAT AAA TTA TCA CTG TCT GAT ATA TCA TCT TTA ATG GAT CAA TTT    2811
Asn Asn Lys Leu Ser Leu Ser Asp Ile Ser Ser Leu Met Asp Gln Phe
    365                 370                 375

CGT TTA AAT CCA TGT ACC ATA AGA AAT ATA TTA TTA TCT TCA GCA ACT    2859
Arg Leu Asn Pro Cys Thr Ile Arg Asn Ile Leu Leu Ser Ser Ala Thr
380                 385                 390                 395

ATA AAA TCA AAA CTA TTA GCG TTA CGG GCA GTA AAA AAC TGG AAA TGT    2907
Ile Lys Ser Lys Leu Leu Ala Leu Arg Ala Val Lys Asn Trp Lys Cys
                400                 405                 410

TAT TCA TTG ACA AAT GTA TCA ATG TAT AAA AAA ATA AAG GGT GTT ATC    2955
Tyr Ser Leu Thr Asn Val Ser Met Tyr Lys Lys Ile Lys Gly Val Ile
            415                 420                 425
```

```
GTA ATG GAT ATG GTT GAT TAT ATA TCT ACT AAC ATT CTT AAA TAC CAT     3003
Val Met Asp Met Val Asp Tyr Ile Ser Thr Asn Ile Leu Lys Tyr His
        430                 435                 440

AAA CAA TTA TAT GAT AAA ATG AGT ACG TTT GAA TAT AAA CGA GAT ATT     3051
Lys Gln Leu Tyr Asp Lys Met Ser Thr Phe Glu Tyr Lys Arg Asp Ile
    445                 450                 455

AAA TCA TGT AAA TGC TCG ATA TGT TCC GAC TCT ATA ACA CAT CAT ATA     3099
Lys Ser Cys Lys Cys Ser Ile Cys Ser Asp Ser Ile Thr His His Ile
460                 465                 470                 475

TAT GAA ACA ACA TCA TGT ATA AAT TAT AAA TCT ACC GAT AAT GAT CTT     3147
Tyr Glu Thr Thr Ser Cys Ile Asn Tyr Lys Ser Thr Asp Asn Asp Leu
                480                 485                 490

ATG ATA GTA TTG TTC AAT CTA ACT AGA TAT TTA ATG CAT GGG ATG ATA     3195
Met Ile Val Leu Phe Asn Leu Thr Arg Tyr Leu Met His Gly Met Ile
            495                 500                 505

CAT CCT AAT CTT ATA AGC GTA AAA GGA TGG GGT CCC CTT ATT GGA TTA     3243
His Pro Asn Leu Ile Ser Val Lys Gly Trp Gly Pro Leu Ile Gly Leu
        510                 515                 520

TTA ACG GGT GAT ATA GGT ATT AAT TTA AAA CTA TAT TCC ACC ATG AAT     3291
Leu Thr Gly Asp Ile Gly Ile Asn Leu Lys Leu Tyr Ser Thr Met Asn
    525                 530                 535

ATA AAT GGG CTA CGG TAT GGA GAT ATT ACG TTA TCT TCA TAC GAT ATG     3339
Ile Asn Gly Leu Arg Tyr Gly Asp Ile Thr Leu Ser Ser Tyr Asp Met
540                 545                 550                 555

AGT AAT AAA TTA GTC TCT ATT ATT AAT ACA CCC ATA TAT GAG TTA ATA     3387
Ser Asn Lys Leu Val Ser Ile Ile Asn Thr Pro Ile Tyr Glu Leu Ile
                560                 565                 570

CCG TTT ACT ACA TGT TGT TCA CTC AAT GAA TAT TAT TCA AAA ATT GTG     3435
Pro Phe Thr Thr Cys Cys Ser Leu Asn Glu Tyr Tyr Ser Lys Ile Val
            575                 580                 585

ATT TTA ATA AAT GTT ATT TTA GAA TAT ATG ATA TCT ATT ATA TTA TAT     3483
Ile Leu Ile Asn Val Ile Leu Glu Tyr Met Ile Ser Ile Ile Leu Tyr
        590                 595                 600

AGA ATA TTG ATC GTA AAA AGA TTT AAT AAC ATT AAA GAA TTT ATT TCA     3531
Arg Ile Leu Ile Val Lys Arg Phe Asn Asn Ile Lys Glu Phe Ile Ser
    605                 610                 615

AAA GTC GTA AAT ACT GTA CTA GAA TCA TCA GGC ATA TAT TTT TGT CAG     3579
Lys Val Val Asn Thr Val Leu Glu Ser Ser Gly Ile Tyr Phe Cys Gln
620                 625                 630                 635

ATG CGT GTA CAT GAA CAA ATT GAA TTG GAA ATA GAT GAG CTC ATT ATT     3627
Met Arg Val His Glu Gln Ile Glu Leu Glu Ile Asp Glu Leu Ile Ile
                640                 645                 650

AAT GGA TCT ATG CCT GTA CAG CTT ATG CAT TTA CTT CTA AAG GTA GCT     3675
Asn Gly Ser Met Pro Val Gln Leu Met His Leu Leu Leu Lys Val Ala
            655                 660                 665

ACC ATA ATA TTA GAG GAA ATC AAA GAA ATA TAACGTATTT TTTCTTTTAA       3725
Thr Ile Ile Leu Glu Glu Ile Lys Glu Ile
        670                 675

ATAAATAAAA ATACTTTTTT TTTTAAACAA GGGGTGCTAC CTTGTCTAAT TGTATCTTGT   3785

ATTTTGGATC TGATGCAAGA TTATTAAATA ATCGTATGAA AAAGTAGTAG ATATAGTTTA   3845
```

TATCGTTACT GGACATGATA TTATGTTTAG TTAATTCTTC TTTGGCATGA ATTCTACACG 3905

TCGGACAAGG TAATGTATCT ATAATGGTAT AAAGCTT 3942

(2) INFORMATION FOR SEQ ID NO:190:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 122 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:190:

Cys Leu Phe Ile Asn Lys Met Gly Gly Ala Ile Ile Glu Tyr Lys Ile
1 5 10 15

Pro Gly Ser Lys Ser Ile Thr Lys Ser Ile Ser Glu Glu Leu Glu Asn
20 25 30

Leu Thr Lys Arg Asp Lys Pro Ile Ser Lys Ile Ile Val Ile Pro Ile
35 40 45

Val Cys Tyr Arg Asn Ala Asn Ser Ile Lys Val Thr Phe Ala Leu Lys
50 55 60

Lys Phe Ile Ile Asp Lys Glu Phe Ser Thr Asn Val Ile Asp Val Asp
65 70 75 80

Gly Lys His Glu Lys Met Ser Met Asn Glu Thr Cys Glu Glu Asp Val
85 90 95

Ala Arg Gly Leu Gly Ile Ile Asp Leu Glu Asp Glu Cys Ile Glu Glu
100 105 110

Asp Asp Val Asp Thr Ser Leu Phe Asn Val
115 120

(2) INFORMATION FOR SEQ ID NO:191:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 75 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:191:

Met Asp Lys Leu Tyr Ala Ala Ile Phe Gly Val Phe Met Thr Ser Lys
1 5 10 15

Asp Asp Asp Phe Asn Asn Phe Ile Glu Val Val Lys Ser Val Leu Thr
20 25 30

Asp Thr Ser Ser Asn His Thr Ile Ser Ser Ser Asn Asn Asn Thr Trp
35 40 45

Ile Tyr Ile Phe Leu Ala Ile Leu Phe Gly Val Met Val Leu Leu Val
50 55 60

Phe Ile Leu Tyr Leu Lys Val Thr Lys Pro Thr
65 70 75

(2) INFORMATION FOR SEQ ID NO:192:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 313 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:192:

```
Met Glu Glu Ala Asp Asn Gln Leu Val Leu Asn Ser Ile Ser Ala Arg
  1               5                  10                  15

Ala Leu Lys Ala Phe Phe Val Ser Lys Ile Asn Asp Met Val Asp Glu
             20                  25                  30

Leu Val Thr Lys Lys Tyr Pro Pro Lys Lys Lys Ser Gln Ile Lys Leu
         35                  40                  45

Ile Asp Thr Arg Ile Pro Ile Asp Leu Ile Asn Gln Gln Phe Val Lys
     50                  55                  60

Arg Phe Lys Leu Glu Asn Tyr Lys Asn Gly Ile Leu Ser Val Leu Ile
 65                  70                  75                  80

Asn Ser Leu Val Glu Asn Asn Tyr Phe Glu Gln Asp Gly Lys Leu Asn
                 85                  90                  95

Ser Ser Asp Ile Asp Glu Leu Val Leu Thr Asp Ile Glu Lys Lys Ile
            100                 105                 110

Leu Ser Leu Ile Pro Arg Cys Ser Pro Leu Tyr Ile Asp Ile Ser Asp
        115                 120                 125

Val Lys Val Leu Ala Ser Arg Leu Lys Lys Ser Ala Lys Ser Phe Thr
    130                 135                 140

Phe Asn Asp His Glu Tyr Ile Ile Gln Ser Asp Lys Ile Glu Glu Leu
145                 150                 155                 160

Ile Asn Ser Leu Ser Arg Asn His Asp Ile Ile Leu Asp Glu Lys Ser
                165                 170                 175

Ser Ile Lys Asp Ser Ile Tyr Ile Leu Ser Asp Asp Leu Leu Asn Ile
            180                 185                 190

Leu Arg Glu Arg Leu Phe Arg Cys Pro Gln Val Lys Asp Asn Thr Ile
        195                 200                 205

Ser Arg Thr Arg Leu Tyr Asp Tyr Phe Thr Arg Val Ser Lys Lys Glu
    210                 215                 220

Glu Ala Lys Ile Tyr Val Ile Leu Lys Asp Leu Lys Ile Ala Asp Ile
225                 230                 235                 240

Leu Gly Ile Glu Thr Val Thr Ile Gly Ser Phe Val Tyr Thr Lys Tyr
                245                 250                 255

Ser Met Leu Ile Asn Ser Ile Ser Ser Asn Val Asp Arg Tyr Ser Lys
            260                 265                 270

Arg Phe His Asp Ser Phe Tyr Glu Asp Ile Ala Glu Phe Ile Lys Asp
        275                 280                 285
```

Asn Glu Lys Ile Asn Val Ser Arg Val Val Glu Cys Leu Ile Val Pro
290 295 300

Asn Ile Asn Ile Glu Leu Leu Thr Glu
305 310

(2) INFORMATION FOR SEQ ID NO:193:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 677 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:193:

Met Pro Ser Tyr Met Tyr Pro Lys Asn Ala Arg Lys Val Ile Ser Lys
1 5 10 15

Ile Ile Ser Leu Gln Leu Asp Ile Lys Lys Leu Pro Lys Lys Tyr Ile
20 25 30

Asn Thr Met Leu Glu Phe Gly Leu His Gly Asn Leu Pro Ala Cys Met
35 40 45

Tyr Lys Asp Ala Val Ser Tyr Asp Ile Asn Asn Ile Arg Phe Leu Pro
50 55 60

Tyr Asn Cys Val Met Val Lys Asp Leu Ile Asn Val Ile Lys Ser Ser
65 70 75 80

Ser Val Ile Asp Thr Arg Leu His Gln Ser Val Leu Lys His Arg Arg
85 90 95

Ala Leu Ile Asp Tyr Gly Asp Gln Asp Ile Ile Thr Leu Met Ile Ile
100 105 110

Asn Lys Leu Leu Ser Ile Asp Asp Ile Ser Tyr Ile Leu Asp Lys Lys
115 120 125

Ile Ile His Val Thr Lys Ile Leu Lys Ile Asp Pro Thr Val Ala Asn
130 135 140

Ser Asn Met Lys Leu Asn Lys Ile Glu Leu Val Asp Val Ile Thr Ser
145 150 155 160

Ile Pro Lys Ser Ser Tyr Thr Tyr Leu Tyr Asn Asn Met Ile Ile Asp
165 170 175

Leu Asp Thr Leu Leu Tyr Leu Ser Asp Ala Phe His Ile Pro Pro Thr
180 185 190

His Ile Ser Leu Arg Ser Leu Arg Asp Ile Asn Arg Ile Ile Glu Leu
195 200 205

Leu Lys Lys Tyr Pro Asn Asn Asn Ile Ile Asp Tyr Ile Ser Asp Ser
210 215 220

Ile Lys Ser Asn Ser Ser Phe Ile His Ile Leu His Met Ile Ile Ser
225 230 235 240

Asn Met Phe Pro Ala Ile Ile Pro Ser Val Asn Asp Phe Ile Ser Thr
245 250 255

Val Val Asp Lys Asp Arg Leu Ile Asn Met Tyr Gly Ile Lys Cys Val
260 265 270

Ala Met Phe Ser Tyr Asp Ile Asn Met Ile Asp Leu Glu Ser Leu Asp
275 280 285

Asp Ser Asp Tyr Ile Phe Ile Glu Lys Asn Ile Ser Ile Tyr Asp Val
290 295 300

Lys Cys Arg Asp Phe Ala Asn Met Ile Arg Asp Lys Val Lys Arg Glu
305 310 315 320

Lys Asn Arg Ile Leu Thr Thr Lys Cys Glu Asp Ile Ile Arg Tyr Ile
325 330 335

Lys Leu Phe Ser Lys Asn Arg Ile Asn Asp Glu Asn Asn Lys Val Glu
340 345 350

Glu Val Leu Ile His Ile Asp Asn Val Ser Lys Asn Asn Lys Leu Ser
355 360 365

Leu Ser Asp Ile Ser Ser Leu Met Asp Gln Phe Arg Leu Asn Pro Cys
370 375 380

Thr Ile Arg Asn Ile Leu Leu Ser Ser Ala Thr Ile Lys Ser Lys Leu
385 390 395 400

Leu Ala Leu Arg Ala Val Lys Asn Trp Lys Cys Tyr Ser Leu Thr Asn
405 410 415

Val Ser Met Tyr Lys Lys Ile Lys Gly Val Ile Val Met Asp Met Val
420 425 430

Asp Tyr Ile Ser Thr Asn Ile Leu Lys Tyr His Lys Gln Leu Tyr Asp
435 440 445

Lys Met Ser Thr Phe Glu Tyr Lys Arg Asp Ile Lys Ser Cys Lys Cys
450 455 460

Ser Ile Cys Ser Asp Ser Ile Thr His His Ile Tyr Glu Thr Thr Ser
465 470 475 480

Cys Ile Asn Tyr Lys Ser Thr Asp Asn Asp Leu Met Ile Val Leu Phe
485 490 495

Asn Leu Thr Arg Tyr Leu Met His Gly Met Ile His Pro Asn Leu Ile
500 505 510

Ser Val Lys Gly Trp Gly Pro Leu Ile Gly Leu Leu Thr Gly Asp Ile
515 520 525

Gly Ile Asn Leu Lys Leu Tyr Ser Thr Met Asn Ile Asn Gly Leu Arg
530 535 540

Tyr Gly Asp Ile Thr Leu Ser Ser Tyr Asp Met Ser Asn Lys Leu Val
545 550 555 560

Ser Ile Ile Asn Thr Pro Ile Tyr Glu Leu Ile Pro Phe Thr Thr Cys
565 570 575

Cys Ser Leu Asn Glu Tyr Tyr Ser Lys Ile Val Ile Leu Ile Asn Val
580 585 590

Ile Leu Glu Tyr Met Ile Ser Ile Ile Leu Tyr Arg Ile Leu Ile Val
595 600 605

```
Lys Arg Phe Asn Asn Ile Lys Glu Phe Ile Ser Lys Val Val Asn Thr
    610                 615                 620

Val Leu Glu Ser Ser Gly Ile Tyr Phe Cys Gln Met Arg Val His Glu
625                 630                 635                 640

Gln Ile Glu Leu Glu Ile Asp Glu Leu Ile Ile Asn Gly Ser Met Pro
                645                 650                 655

Val Gln Leu Met His Leu Leu Leu Lys Val Ala Thr Ile Ile Leu Glu
            660                 665                 670

Glu Ile Lys Glu Ile
        675
```

(2) INFORMATION FOR SEQ ID NO:194:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 64 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:194:

```
Lys Leu Tyr Thr Ile Ile Asp Thr Leu Pro Cys Pro Thr Cys Arg Ile
  1               5                  10                  15

His Ala Lys Glu Glu Leu Thr Lys His Asn Ile Met Ser Ser Asn Asp
             20                  25                  30

Ile Asn Tyr Ile Tyr Tyr Phe Phe Ile Arg Leu Phe Asn Asn Leu Ala
         35                  40                  45

Ser Asp Pro Lys Tyr Lys Ile Gln Leu Asp Lys Val Ala Pro Leu Val
     50                  55                  60
```

(2) INFORMATION FOR SEQ ID NO:195:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 583 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox virus
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: S-SPV-001

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..583

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:195:

```
A AGC TTA AGA AAG AAT GTA GGG AAC GAA GAA TAT AGA ACC AAA GAT        46
  Ser Leu Arg Lys Asn Val Gly Asn Glu Glu Tyr Arg Thr Lys Asp
   1               5                  10                  15

TTA TTT ACT GCA TTA TGG GTA CCT GAT TTA TTT ATG GAA CGC GTA GAA      94
Leu Phe Thr Ala Leu Trp Val Pro Asp Leu Phe Met Glu Arg Val Glu
                 20                  25                  30

AAA GAT GAA GAA TGG TCT CTA ATG TGT CCA TGC GAA TGT CCA GGA TTA     142
Lys Asp Glu Glu Trp Ser Leu Met Cys Pro Cys Glu Cys Pro Gly Leu
             35                  40                  45

TGC GAT GTA TGG GGG AAT GAT TTT AAC AAA TTA TAT ATA GAA TAC GAA     190
Cys Asp Val Trp Gly Asn Asp Phe Asn Lys Leu Tyr Ile Glu Tyr Glu
         50                  55                  60

ACA AAG AAA AAA ATT AAA GCG ATC GCT AAA GCA AGA AGT TTA TGG AAA     238
Thr Lys Lys Lys Ile Lys Ala Ile Ala Lys Ala Arg Ser Leu Trp Lys
     65                  70                  75

TCT ATT ATC GAG GCT CAA ATA GAA CAA GGA ACG CCG TAT ATA CTA TAT     286
Ser Ile Ile Glu Ala Gln Ile Glu Gln Gly Thr Pro Tyr Ile Leu Tyr
 80                  85                  90                  95

AAA GAT TCT TGT AAT AAA AAA TCC AAT CAA AGC AAT TTG GGA ACA ATT     334
Lys Asp Ser Cys Asn Lys Lys Ser Asn Gln Ser Asn Leu Gly Thr Ile
                100                 105                 110

AGA TCG AGT AAT CTC TGT ACA GAG ATT ATA CAA TTT AGT AAC GAG GAT     382
Arg Ser Ser Asn Leu Cys Thr Glu Ile Ile Gln Phe Ser Asn Glu Asp
            115                 120                 125

GAA GTT GCT GTA TGT AAT CTA GGA TCT ATT TCG TGG AGT AAA TTT GTT     430
Glu Val Ala Val Cys Asn Leu Gly Ser Ile Ser Trp Ser Lys Phe Val
        130                 135                 140

AAT AAT AAC GTA TTT ATG TTC GAC AAG TTG AGA ATA ATT ACG AAA ATA     478
Asn Asn Asn Val Phe Met Phe Asp Lys Leu Arg Ile Ile Thr Lys Ile
    145                 150                 155

CTA GTT AAA AAT CTA AAT AAA ATA ATA GAT ATC AAT TAT TAT CCA GTG     526
Leu Val Lys Asn Leu Asn Lys Ile Ile Asp Ile Asn Tyr Tyr Pro Val
160                 165                 170                 175

ATA GAA TCG TCT AGA TCT AAT AAG AAA CAT AGA CCC ATA GGT ATC GGG     574
Ile Glu Ser Ser Arg Ser Asn Lys Lys His Arg Pro Ile Gly Ile Gly
                180                 185                 190

GTT CAG GGT                                                         583
Val Gln Gly
```

(2) INFORMATION FOR SEQ ID NO:196:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 194 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:196:

```
Ser Leu Arg Lys Asn Val Gly Asn Glu Glu Tyr Arg Thr Lys Asp Leu
 1               5                  10                  15
```

```
Phe Thr Ala Leu Trp Val Pro Asp Leu Phe Met Glu Arg Val Glu Lys
             20                  25                  30

Asp Glu Glu Trp Ser Leu Met Cys Pro Cys Glu Cys Pro Gly Leu Cys
         35                  40                  45

Asp Val Trp Gly Asn Asp Phe Asn Lys Leu Tyr Ile Glu Tyr Glu Thr
     50                  55                  60

Lys Lys Lys Ile Lys Ala Ile Ala Lys Ala Arg Ser Leu Trp Lys Ser
 65                  70                  75                  80

Ile Ile Glu Ala Gln Ile Glu Gln Gly Thr Pro Tyr Ile Leu Tyr Lys
                 85                  90                  95

Asp Ser Cys Asn Lys Lys Ser Asn Gln Ser Asn Leu Gly Thr Ile Arg
            100                 105                 110

Ser Ser Asn Leu Cys Thr Glu Ile Ile Gln Phe Ser Asn Glu Asp Glu
        115                 120                 125

Val Ala Val Cys Asn Leu Gly Ser Ile Ser Trp Ser Lys Phe Val Asn
    130                 135                 140

Asn Asn Val Phe Met Phe Asp Lys Leu Arg Ile Ile Thr Lys Ile Leu
145                 150                 155                 160

Val Lys Asn Leu Asn Lys Ile Ile Asp Ile Asn Tyr Tyr Pro Val Ile
                165                 170                 175

Glu Ser Ser Arg Ser Asn Lys Lys His Arg Pro Ile Gly Ile Gly Val
            180                 185                 190

Gln Gly
```

(2) INFORMATION FOR SEQ ID NO:197:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:197:

```
ACAGGAAACA GCTATGACCA TGATTACGAA TTCGAGCTCG CCCGGGGATC T                51
```

(2) INFORMATION FOR SEQ ID NO:198:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 138 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:198:

GTATAGCGGC CGCCTGCAGG TCGACTCTAG ATTTTTTTTT TTTTTTTTTT TGGCATATAA 60

ATAGATCTGT ATCCTAAAAT TGAATTGTAA TTATCGATAA TAAATGAATT CGATGGCTGT 120

GCCTGCAAGC CCACAGCA 138

(2) INFORMATION FOR SEQ ID NO:199:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 120 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:199:

CTTAGCCCCA AACGCACCTC AGATCCATAA TTAATTAATT TTTATCCCGG CGCGCCTCGA 60

CTCTAGAATT TCATTTTGTT TTTTTCTATG CTATAAATGA ATTCGGATCC CGTCGTTTTA 120

(2) INFORMATION FOR SEQ ID NO:200:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 116 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:200:

GAAATCCAGC TGAGCGCCGG TCGCTACCAT TACCAGTTGG TCTGGTGTCA AAAAGATCCA 60

TAATTAATTA ACCCGGGTCG AGGCGCGCCG GGTCGACCTG CAGGCGGCCG CTATAC 116

(2) INFORMATION FOR SEQ ID NO:201:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:201:

TAATGTATCT ATAATGGTAT AAAGCTTGTA TTCTATAGTG TCACCTAAAT C 51

(2) INFORMATION FOR SEQ ID NO:202:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:202:

ACAGGAAACA GCTATGACCA TGATTACGAA TTCGAGCTCG CCCGGGGATC T 51

(2) INFORMATION FOR SEQ ID NO:203:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 141 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:203:

GTATAGCGGC CGCCTGCAGG TCGACTCTAG ATTTTTTTTT TTTTTTTTTT TGGCATATAA 60

ATAGATCTGT ATCCTAAAAT TGAATTGTAA TTATCGATAA TAAATGAATT CCATGTGCTG 120

CCTCACCCCT GTGCTGGCGC T 141

(2) INFORMATION FOR SEQ ID NO:204:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 120 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:204:

TCGCCCGCCT CTGACGCCCC GGATCCATAA TTAATTAATT TTTATCCCGG CGCGCCTCGA 60

CTCTAGAATT TCATTTTGTT TTTTTCTATG CTATAAATGA ATTCGGATCC CGTCGTTTTA 120

(2) INFORMATION FOR SEQ ID NO:205:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 116 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:205:

GAAATCCAGC TGAGCGCCGG TCGCTACCAT TACCAGTTGG TCTGGTGTCA AAAAGATCCA 60

TAATTAATTA ACCCGGGTCG AGGCGCGCCG GGTCGACCTG CAGGCGGCCG CTATAC 116

(2) INFORMATION FOR SEQ ID NO:206:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:206:

TAATGTATCT ATAATGGTAT AAAGCTTGTA TTCTATAGTG TCACCTAAAT C 51

(2) INFORMATION FOR SEQ ID NO:207:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:207:

CAAGGAATGG TGCATGCCCG TTCTTATCAA TAGTTTAGTC GAAAA 45

(2) INFORMATION FOR SEQ ID NO:208:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:208:

TATATAAGCA CTTATTTTTG TTAGTATAAT AACACAATGC CAGATCCCGT CGTTTTA 57

(2) INFORMATION FOR SEQ ID NO:209:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 249 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:209:

TCCAGCTGAG CGCCGGTCGC TACCATTACC AGTTGGTCTG GTGTCAAAAA GATCCATAAT 60

TAATTAACCA GCGGCCGCCT GCAGGTCGAC TCTAGATTTT TTTTTTTTTT TTTTTTGGCA 120

TATAAATAGA TCTGTATCCT AAAATTGAAT TGTAATTATC GATAATAAAT GAATTCGGAT 180

CCATAATTAA TTAATTTTTA TCCCGGCGCG CCGGGTCGAC CTGCAGGCGG CCGCTGGGTC 240

GACAAAGAT 249

(2) INFORMATION FOR SEQ ID NO:210:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:210:

CAAAAGTCGT AAATACTGTA CTAGAAGCTT GGCGTAATCA TGGTC 45

(2) INFORMATION FOR SEQ ID NO:211:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:211:

CGACGGATCC GAGGTGCGTT TGGGGCTAAG TGC 33

(2) INFORMATION FOR SEQ ID NO:212:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:212:

CCACGGATCC AGCACAACGC GAGTCCCACC ATGGCT 36

(2) INFORMATION FOR SEQ ID NO:213:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:213:

CCACGAATTC GATGGCTGTG CCTGCAAGCC CACAG 35

(2) INFORMATION FOR SEQ ID NO:214:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:214:

CGAAGATCTG AGGTGCGTTT GGGGCTAAGT GC 32

(2) INFORMATION FOR SEQ ID NO:215:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:215:

CGCAGGATCC GGGGCGTCAG AGGCGGGCGA GGTG 34

(2) INFORMATION FOR SEQ ID NO:216:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:216:

GAGCGGATCC TGCAGGAGGA GACACAGAGC TG 32

(2) INFORMATION FOR SEQ ID NO:217:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:217:

GCGCGAATTC CATGTGCTGC CTCACCCCTG TG 32

(2) INFORMATION FOR SEQ ID NO:218:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:218:

CGCAGGATCC GGGGCGTCAG AGGCGGGCGA GGTG 34

(2) INFORMATION FOR SEQ ID NO:219:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:219:

GGGGAATTCA ATGCAACCCA CCGCGCCGCC CC 32

(2) INFORMATION FOR SEQ ID NO:220:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:220:

GGGGATCCT AGGGCGCGCC CGCCGGCTCG CT 32

(2) INFORMATION FOR SEQ ID NO:221:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5785 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: N

(iv) ANTI-SENSE: N

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:221:

AAGCTTAAGA AAGAATGTAG GGAACGAAGA ATATAGAACC AAAGATTTAT TTACTGCATT
60

ATGGGTACCT GATTTATTTA TGGAACGCGT AGAAAAAGAT GAAGAATGGT CTCTAATGTG
120

TCCATGCGAA TGTCCAGGAT TATGCGATGT ATGGGGGAAT GATTTTAACA AATTATATAT
180

AGAATACGAA ACAAAGAAAA AAATTAAAGC GATCGCTAAA GCAAGAAGTT TATGGAAATC
240

TATTATCGAG GCTCAAATAG AACAAGGAAC GCCGTATATA CTATATAAAG ATTCTTGTAA
300

TAAAAAATCC AATCAAAGCA ATTTGGGAAC AATTAGATCG AGTAATCTCT GTACAGAGAT
360

TATACAATTT AGTAACGAGG ATGAAGTTGC TGTATGTAAT CTAGGATCTA TTTCGTGGAG
420

TAAATTTGTT AATAATAACG TATTTATGTT CGACAAGTTG AGAATAATTA CGAAAATACT
480

AGTTAAAAAT CTAAATAAAA TAATAGATAT CAATTATTAT CCAGTGATAG AATCGTCTAG
540

ATCTAATAAG AAACATAGAC CCATAGGTAT CGGTGTTCAG GGTTTGGCTG ATGTGTTTAT
600

ATTATTGGGC TATGCATTCG ATAGCGAAGA AGCAAAAATA TTAAATATAC AAATTTCCGA
660

AACAATATAT TATGCCGCAC TAGAATCTAG TTGCGAACTA GCTAAAATTT ACGGACCTTA
720

TGAGACATAT AACGATTCTC CAGCGAGTAA AGGTATTCTA CAATATGATA TGTGGTTAAA
780

GAACCCAACA GATTTATGGG ATTGGAATGA ACTAAAAAAG AGAATTAATA CACATGGATT
840

GAGAAATAGC CTTCTAATAG CACCAATGCC TACTGCATCT ACATCTCAAA TATTAAGTAA
900

TAATGAGTCC ACCGAACCAT ATACTAGCAA TATATATACA AGAAGAGTAT TATCTGGAGA
960

TTTTCAGGTT GTAAATCCAC ACCTATTGAG AGAACTAATA AGTAGAAATA TGTGGAATAA
1020

TGACATAAAG AATACAATTG TGTTACATAA TGGTTCTATT CAACATTTAG ATTTACCAGA
1080

TAATATAAAA CCAATATATA AAACGGTTTG GGAGATATCT CCAAAATGTA TTTTAGAAAT
1140

GGCAGCCGAC AGAGGTGCGT TTATAGATCC AAGTCAATCA ATGACAATAT ATATAGATAA
1200

TCCTACATAC GCAAAACTGA CCAGTATGCA TTTTTACGGA TGGAGATTGG GGCTAAAAAC
1260

TGGGATGTAT TATATGAGAA CAAAATCGGC ATCAAATCCT ATAAAATTCA CAGTTGAGTG
1320

TAGTAATTGT TCTGCATAAT TTTTATAAAA ATGAAATACT ATCTCATGTA TCTTAATATA
1380

TTAAAAATGC GTAAAAGTGG CATTCCAAAA CAACCCGTTC CCAAAAAAGA TTATGTTCAA
1440

ACTGATAATA ATAAAAAACA ACAAATAACA ACGTGTTCAG AAGTCGTTGA GTATCTTAAA
1500

TCACTAAGTA AGAGCACCGA AAAATGTATA GAAAATGTAA TATTAACGCC TTCTCAATAT
1560

CCTTCTTGTT CATCGATAAC TATTAATTTA ACAGACTATC TATCATCTAA AATGACATCT
1620

ACATATATAG CATTAGAAGG TGAGTCTAAA ATATACAAGA ATAAAAAGAA TGAAAGTAGA
1680

TCGTTAGATC AATATTTTTT AAAAATACGA CTTACTGCAG CAAGTCCTAT AATGTATCAA
1740

TTATTAGATT GTATATATTC TAATATTAGA GATAATAAAC ATATACCCCC TTCCTTATCA
1800

AATATATCTA TATCGGACTT AGAAGAGAAA ACGCTTAACC AGGGGTGTTT GTTCATTAAT
1860

AAGATGGGTG GAGCTATTAT AGAATACAAG ATACCTGGTT CCAAATCTAT AACAAAATCT
1920

ATTTCCGAAG AACTAGAAAA TTTAACAAAG CGAGATAAAC AAATATCTAA AATTATAGTT
1980

ATTCCTATTG TATGTTACAG AAATGCAAAT AGTATAAAGG TTACATTTGC ACTAAAAAAG
2040

TTTATCATAG ATAAGGAGTT TAGTACAAAT GTAATAGACG TAGATGGTAA ACATGAAAAA
2100

ATGTCCATGA ATGAAACATG CGAAGAGGAT GTTGCTAGAG GATTGGGAAT TATAGATCTT
2160

GAAGATGAAT GCATAGAGGA AGATGATGTC GATACGTCAT TATTTAATGT ATAAATGGAT
2220

AAATTGTATG CGGCAATATT CGGCGTTTTT ATGACATCTA AAGATGATGA TTTTAATAAC
2280

TTTATAGAAG TTGTAAAATC TGTATTAACA GATACATCAT CTAATCATAC AATATCGTCG
2340

TCCAATAATA ATACATGGAT ATATATATTT CTAGCGATAT TATTTGGTGT TATGGTATTA
2400

TTAGTTTTTA TTTTGTATTT AAAAGTTACT AAACCAACTT AAATGGAGGA AGCAGATAAC
2460

CAACTCGTTT TAAATAGTAT TAGTGCTAGA GCATTAAAGG CATTTTTTGT ATCTAAAATT
2520

AATGATATGG TCGATGAATT AGTTACCAAA AAATATCCAC CAAAGAAGAA ATCACAAATA
2580

AAACTCATAG ATACACGAAT TCCTATTGAT CTTATTAATC AACAATTCGT TAAAAGATTT
2640

AAACTAGAAA ATTATAAAAA TGGAATTTTA TCCGTTCTTA TCAATAGTTT AGTCGAAAAT
2700

AATTACTTTG AACAAGATGG TAAACTTAAT AGCAGTGATA TTGATGAATT AGTGCTCACA
2760

GACATAGAGA AAAAGATTTT ATCGTTGATT CCTAGATGTT CTCCTCTTTA TATAGATATC
2820

AGTGACGTTA AAGTTCTCGC ATCTAGGTTA AAAAAGTGCT AAATCATTTA CGTTTAATGA
2880

TCATGAATAT ATTATACAAT CTGATAAAAT AGAGGAATTA ATAAATAGTT TATCTAGAAA
2940

CCATGATATT ATACTAGATG AAAAAAGTTC TATTAAAGAC AGCATATATA TACTATCTGA
3000

TGATCTTTTG AATATACTTC GTGAAAGATT ATTTAGATGT CCACAGGTTA AAGATAATAC
3060

TATTTCTAGA ACACGTCTAT ATGATTATTT TACTAGAGTG TCAAAGAAAG AAGAAGCGAA
3120

AATATACGTT ATATTGAAAG ATTTAAAGAT TGCTGATATA CTCGGTATCG AAACAGTAAC
3180

GATAGGATCA TTTGTATATA CGAAATATAG CATGTTGATT AATTCAATTT CGTCTAATGT
3240

TGATAGATAT TCAAAAAGGT TCCATGACTC TTTTTATGAA GATATTGCGG AATTTATAAA
3300

GGATAATGAA AAAATTAATG TATCCAGAGT TGTTGAATGC CTTATCGTAC CTAATATTAA
3360

TATAGAGTTA TTAACTGAAT AAGTATATAT AAATGATTGT TTTTATAATG TTTGTTATCG
3420

CATTTAGTTT TGCTGTATGG TTATCATATA CATTTTTAAG GCCGTATATG ATAAATGAAA
3480

ATATATAAGC ACTTATTTTT GTTAGTATAA TAACACAATG CCGTCGTATA TGTATCCGAA
3540

GAACGCAAGA AAAGTAATTT CAAAGATTAT ATCATTACAA CTTGATATTA AAAAACTTCC
3600

TAAAAAATAT ATAAATACCA TGTTAGAATT TGGTCTACAT GGAAATCTAC CAGCTTGTAT
3660

GTATAAAGAT GCCGTATCAT ATGATATAAA TAATATAAGA TTTTTACCTT ATAATTGTGT
3720

TATGGTTAAA GATTTAATAA ATGTTATAAA ATCATCATCT GTAATAGATA CTAGATTACA
3780

TCAATCTGTA TTAAAACATC GTAGAGCGTT AATAGATTAC GGCGATCAAG ACATTATCAC
3840

TTTAATGATC ATTAATAAGT TACTATCGAT AGATGATATA TCCTATATAT TAGATAAAAA
3900

AATAATTCAT GTAACAAAAA TATTAAAAAT AGACCCTACA GTAGCCAATT CAAACATGAA
3960

ACTGAATAAG ATAGAGCTTG TAGATGTAAT AACATCAATA CCTAAGTCTT CCTATACATA
4020

TTTATATAAT AATATGATCA TTGATCTCGA TACATTATTA TATTTATCCG ATGCATTCCA
4080

CATACCCCCC ACACATATAT CATTACGTTC ACTTAGAGAT ATAAACAGGA TTATTGAATT
4140

GCTTAAAAAA TATCCGAATA ATAATATTAT TGATTATATA TCCGATAGCA TAAAATCAAA
4200

TAGTTCATTC ATTCACATAC TTCATATGAT AATATCAAAT ATGTTTCCTG CTATAATCCC
4260

TAGTGTAAAC GATTTTATAT CTACCGTAGT TGATAAAGAT CGACTTATTA ATATGTATGG
4320

GATTAAGTGT GTTGCTATGT TTTCGTACGA TATAAACATG ATCGATTTAG AGTCATTAGA
4380

TGACTCAGAT TACATATTTA TAGAAAAAAA TATATCTATA TACGACGTTA AATGTAGAGA
4440

TTTTGCGAAT ATGATTAGAG ATAAGGTTAA AAGAGAAAAG AATAGAATAT TAACTACGAA
4500

ATGTGAAGAT ATTATAAGAT ATATAAAATT ATTCAGTAAA AATAGAATAA ACGATGAAAA
4560

TAATAAGGTG GAGGAGGTGT TGATACATAT TGATAATGTA TCTAAAAATA ATAAATTATC
4620

ACTGTCTGAT ATATCATCTT TAATGGATCA ATTTCGTTTA AATCCATGTA CCATAAGAAA
4680

TATATTATTA TCTTCAGCAA CTATAAAATC AAAACTATTA GCGTTACGGG CAGTAAAAAA
4740

CTGGAAATGT TATTCATTGA CAAATGTATC AATGTATAAA AAAATAAAGG GTGTTATCGT
4800

AATGGATATG GTTGATTATA TATCTACTAA CATTCTTAAA TACCATAAAC AATTATATGA
4860

TAAAATGAGT ACGTTTGAAT ATAAACGAGA TATTAAATCA TGTAAATGCT CGATATGTTC
4920

CGACTCTATA ACACATCATA TATATGAAAC AACATCATGT ATAAATTATA AATCTACCGA
4980

TAATGATCTT ATGATAGTAT TGTTCAATCT AACTAGATAT TTAATGCATG GGATGATACA
5040

TCCTAATCTT ATAAGCGTAA AAGGATGGGG TCCCCTTATT GGATTATTAA CGGGTGATAT
5100

AGGTATTAAT TTAAAACTAT ATTCCACCAT GAATATAAAT GGGCTACGGT ATGGAGATAT
5160

TACGTTATCT TCATACGATA TGAGTAATAA ATTAGTCTCT ATTATTAATA CACCCATATA
5220

TGAGTTAATA CCGTTTACTA CATGTTGTTC ACTCAATGAA TATTATTCAA AAATTGTGAT
5280

TTTAATAAAT GTTATTTTAG AATATATGAT ATCTATTATA TTATATAGAA TATTGATCGT
5340

AAAAAGATTT AATAACATTA AAGAATTTAT TTCAAAAGTC GTAAATACTG TACTAGAATC
5400

ATCAGGCATA TATTTTTGTC AGATGCGTGT ACATGAACAA ATTGAATTGG AAATAGATGA
5460

GCTCATTATT AATGGATCTA TGCCTGTACA GCTTATGCAT TTACTTCTAA AGGTAGCTAC
5520

CATAATATTA GAGGAAATCA AAGAAATATA ACGTATTTTT TCTTTTAAAT AAATAAAAAT
5580

ACTTTTTTTT TTAAACAAGG GGTGCTACCT TGTCTAATTG TATCTTGTAT TTTGGATCTG
5640

ATGCAAGATT ATTAAATAAT CGTATGAAAA AGTAGTAGAT ATAGTTTATA TCGTTACTGG
5700

ACATGATATT ATGTTTAGTT AATTCTTCTT TGGCATGAAT TCTACACGTC GGACAAGGTA
5760

ATGTATCTAT AATGGTATAA AGCTT
5785

(2) INFORMATION FOR SEQ ID NO:222:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 722 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: N

(iv) ANTI-SENSE: N

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:222:

TTTTGATTTT ACGCCATTAT ACTGTTCTGT AGATGCAAAT AATGAAGATG TGTTCTTATT
60

TACTAGAGAG ATGCAGACCC TATATTATCA CAGTATTTGG TGAACGTGTA TACTAACAGC
120

TTCAATAATC ATAATCCCCC ATATTATATA ACTATTAAAT TATGATATAG ATATAAATAC
180

TATCCAAAAT ACATTATTTA AACTGGAACA AGATATTATT AACTCTACCA TAGATACTTA
240

CTATTACAAT AATCTTGTTA AAAAAGAACA TTTTATAAAA TTATTTCTAG CCTACATAGT
300

TAAGAGGTAT GAAAAAAATA TAGGAATATT ATTTCTTGAT TATCCCACTC TTGGTGAATA
360

TTTCGTGAAA TTTATAGATA CGTGTATGAT GGAAATATTT GAGATGAAAT CAGATAAGGT
420

GGTAAACGGA TATATATTCT ATTATATTTA CGAATAAGTA TATTCCTATC CCATATATAA
480

CGTGTAAAAA GCTAAAGAAA TACGAATCCT TTGTTGTATA TGGAACCGAA ATAAAATCAA
540

TAATAAAATC TTCAAAGATT AGATATGCGA GTGTTATAAA AGTAACGGAG TATATCACAT
600

CTATCTGTTC GGAAGAAACT AGTTTATGGA ACAGCATCCC AATTGAGATA AAACATAAGA
660

TTATTAATAA TATAAACAAT CATGATATGT ATATATTATA TAAAAATAGA AAAAAAAAAT
720

AA
722

(2) INFORMATION FOR SEQ ID NO:223:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 234 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: N

(iv) ANTI-SENSE: N

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:223:

AAACAATGCG CTTTAATATC AAACATGCAG GTGGAATAGG ATTGTCGATA AGTAATATAC 60

GAGCTAAGGG TACTTATATA TCCGGTATAA ACGGCAAATC TATGGTATAG TACCTATGTT 120

AAGAATATAT AATAACACAG TTAGATATAT TAATCAGGGA GGTGATAAAA GACCAGGAGC 180

AATGTCGATT TATATAGAAC CATGGCACGC TGATATATTC GATTTTCTAA GCTT 234

(2) INFORMATION FOR SEQ ID NO:224:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1025 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: N

(iv) ANTI-SENSE: N

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:224:

GGTTGCTCCT AACTTAATAA GATAATCCAC CAAGATAGTT TTATCCGTGG TAGATGCATA 60

CACAACAGGA GAATATCCTA ATTTATCTCT ATAGTTTATG GTTGTGATAT CTATAGTATA 120

TGGGACCGCC GAAAAACATG TATAATCGTC GTGACAATAG TTTAACATCG TGTTTAATAT 180

CGACATCATT TCATCATTTT TATTATATTC ATGTTTTATA TGCGAACAAA GCAAATTCAA 240

TATATTTAAA TTAGTGTTAT TGATGTGTCT AATTGTAAAT ATATGAATAG GATTCTTCAG 300

ACTATTATTT AGTTTACATA CATCAAATCC TTTTCTTATT AAAAACTCAA CAACTTTATA 360

ATCTATATTC TCATTACCAA GGTATTTATG CAATATGGTG TCTCCACATC TATGTACACT 420

GTTAATGTCA CCACCATGAT AAATAAGAAA CTTTATTACT TTAATTGTAA CATTCGTATT 480

AAATGTAAAA TAACAATGAA ATGGTGTTTT ATCATATATA GATATCCCAT TTAAATTAGC 540

ACCTTTATTA AGCAGTAATA ATACAATTTC TTTCAACTCT TTTAATTTAA ATACGTGCAA 600

CGATGAACTT AAAAATGTAG CTAACATATC AGTGGCTATA TTATCATCCT GTTTTATATT 660

TGATATTATT CTTCTTATAT TATCCATTTC CTTCTTACAA ACTATTTAAA CGATAACCAA 720

AATGTATTCA TGGGCTACTA ATAATAGCCA CATTACTAGA AAAAAAATTT TTTTTCAATA
780

TTATGACATT ATTACTTAAG TATTATTGAT AAGTCCTTCA TTGTTAAATG TAATAATATA
840

TATCGTTGTA TTTCTATAGG AATCCTCATC CAGTAACTAT GTTTCTTGCA GTGCTTCATA
900

ATTACATAAA TCGCTTTATC AATGTTAGAA TAATACATAT ATGTATTTTT GATAATATTT
960

TCTATATGTG ATCCATACAT TACTAAATTT TTTAATCTTA AAAAATTATC ATAATTGAGA
1020

AGCTT
1025

(2) INFORMATION FOR SEQ ID NO:225:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 305 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: N

(iv) ANTI-SENSE: N

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:225:

AAGCTTGGAT GAGCAATAAG AGTATACAAA ATTTAGTGTT TCAATTCGCT CATGGATCAG
60

AAGTAGAATA TATAGGTCAA TACGATATGA GATTTTTAAA TAATATACCT ATTCATGATA
120

AGTTTGATGT GTTTTTAAAT AAGCACATAC TATCGTATGT ACTTAGAGAT AAAATAAAGA
180

AATCAGACCA CAGATATGTA ATGTTTGGAT TTTGGTTATT TATCTCATTG GAAATGTGTT
240

ATATTCGATA AGGAACATCA TATGTCTGTT TCTATGATTC AGGAGGAATT ACCAAACGAA
300

TTCCA
305

(2) INFORMATION FOR SEQ ID NO:226:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1721 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: N

(iv) ANTI-SENSE: N

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1721
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:226:

```
ATG AAT TCG GAT CCG GCA ATA CTA TTA GTC TTG CTA TGT ACA TTT ACA
48
Met Asn Ser Asp Pro Ala Ile Leu Leu Val Leu Leu Cys Thr Phe Thr
  1               5                  10                  15

ACC GCA AAT GCA GAC ACA TTA TGT ATA GGT TAC CAT GCA AAT AAT TCA
96
Thr Ala Asn Ala Asp Thr Leu Cys Ile Gly Tyr His Ala Asn Asn Ser
             20                  25                  30

ACT GAC ACT GTT GAC ACA GTA CTA GAA AAG AAT GTA ACA GTA ACA CAC
144
Thr Asp Thr Val Asp Thr Val Leu Glu Lys Asn Val Thr Val Thr His
         35                  40                  45

TCT GTT AAC CTT CTA GAA GAC AGA CAT AAC GGG AAA CTA TGT AAA CTA
192
Ser Val Asn Leu Leu Glu Asp Arg His Asn Gly Lys Leu Cys Lys Leu
     50                  55                  60

AGA GGG GTA GCC CCA TTG CAT TTG GGT AAA TGT AAC ATT GCT GGA TGG
240
Arg Gly Val Ala Pro Leu His Leu Gly Lys Cys Asn Ile Ala Gly Trp
 65                  70                  75                      80

CTC CTG GGA AAC CCA GAG TGT GAA TTA CTA TTC ACA GCA AGC TCA TGG
288
Leu Leu Gly Asn Pro Glu Cys Glu Leu Leu Phe Thr Ala Ser Ser Trp
                 85                  90                  95

TCT TAC ATT GTG GAA ACA TCT AAT TCA GAC AAT GGG ACA TGT TAC CCA
336
Ser Tyr Ile Val Glu Thr Ser Asn Ser Asp Asn Gly Thr Cys Tyr Pro
            100                 105                 110

GGA GAT TTC ATC AAT TAT GAA GAG CTA AGA GAG CAG TTG AGC TCA GTG
384
Gly Asp Phe Ile Asn Tyr Glu Glu Leu Arg Glu Gln Leu Ser Ser Val
        115                 120                 125

TCA TCA TTT GAA AGA TTT GAG ATA TTC CCC AAG GCA AGT TCA TGG CCC
432
Ser Ser Phe Glu Arg Phe Glu Ile Phe Pro Lys Ala Ser Ser Trp Pro
    130                 135                 140

AAT CAT GAA ACG AAC ATA GGT GTG ACG GCA GCA TGT CCT TAT GCT GGA
480
Asn His Glu Thr Asn Ile Gly Val Thr Ala Ala Cys Pro Tyr Ala Gly
145                 150                 155                     160

GCA AAC AGC TTC TAC AGA AAC TTA ATA TGG CTG GTA AAA AAA GGA AAT
528
Ala Asn Ser Phe Tyr Arg Asn Leu Ile Trp Leu Val Lys Lys Gly Asn
                165                 170                 175

TCA TAC CCA AAG CTC AGC AAA TCC TAT ATT AAC AAT AAG GAG AAG GAA
576
```

```
Ser Tyr Pro Lys Leu Ser Lys Ser Tyr Ile Asn Asn Lys Glu Lys Glu
            180                 185                 190

GTC CTC GTG CTA TGG GGC ATT CAC CAT CCA CCT ACC AGT ACT GAC CAA
624
Val Leu Val Leu Trp Gly Ile His His Pro Pro Thr Ser Thr Asp Gln
        195                 200                 205

CAA AGT CTC TAC CAG AAT GCA GAT GCC TAT GTT TTT GTG GGG TCA TCA
672
Gln Ser Leu Tyr Gln Asn Ala Asp Ala Tyr Val Phe Val Gly Ser Ser
    210                 215                 220

AAA TAC AAC AAG AAA TTC AAG CCA GAA ATA GCA ACA AGA CCC AAG GTG
720
Lys Tyr Asn Lys Lys Phe Lys Pro Glu Ile Ala Thr Arg Pro Lys Val
225                 230                 235                 240

AGA GGT CAA GCA GGG AGA ATG AAC TAT TAC TGG ACG CTA GTA AAG CCT
768
Arg Gly Gln Ala Gly Arg Met Asn Tyr Tyr Trp Thr Leu Val Lys Pro
                245                 250                 255

GGA GAC ACA ATA ACA TTC GAA GCA ACT GGA AAT CTA GTG GTA CCA AGA
816
Gly Asp Thr Ile Thr Phe Glu Ala Thr Gly Asn Leu Val Val Pro Arg
            260                 265                 270

TAT GCC TTC GCA ATG AAA AGA GGT TCT GGA TCT GGT ATT ATC ATT TCA
864
Tyr Ala Phe Ala Met Lys Arg Gly Ser Gly Ser Gly Ile Ile Ile Ser
        275                 280                 285

GAT ACA CCA GTC CAC GAT TGT AAT ACG ACT TGT CAA ACA CCC AAA GGT
912
Asp Thr Pro Val His Asp Cys Asn Thr Thr Cys Gln Thr Pro Lys Gly
    290                 295                 300

GCT ATA AAC ACC AGC CTT CCA TTT CAG AAT ATA CAT CCA GTC ACA ATT
960
Ala Ile Asn Thr Ser Leu Pro Phe Gln Asn Ile His Pro Val Thr Ile
305                 310                 315                 320

GGA GAA TGT CCA AAA TAT GTC AAA AGC ACA AAA TTG AGA ATG GCT ACA
1008
Gly Glu Cys Pro Lys Tyr Val Lys Ser Thr Lys Leu Arg Met Ala Thr
                325                 330                 335

GGA TTA AGG AAT ATC CCG TCT ATT CAA TCT AGA GGC CTG TTT GGA GCC
1056
Gly Leu Arg Asn Ile Pro Ser Ile Gln Ser Arg Gly Leu Phe Gly Ala
            340                 345                 350

ATT GCT GGC TTT ATT GAG GGG GGA TGG ACA GGA ATG ATA GAT GGC TGG
1104
Ile Ala Gly Phe Ile Glu Gly Gly Trp Thr Gly Met Ile Asp Gly Trp
        355                 360                 365

TAC GGT TAT CAC CAT CAG AAT GAG CAG GGA TCA GGA TAT GCA GCC GAC
1152
Tyr Gly Tyr His His Gln Asn Glu Gln Gly Ser Gly Tyr Ala Ala Asp
    370                 375                 380

CGA AAG AGC ACA CAG AAT GCC ATT GAC GGG ATC ACT AAC AAA GTA AAC
1200
Arg Lys Ser Thr Gln Asn Ala Ile Asp Gly Ile Thr Asn Lys Val Asn
385                 390                 395                 400
```

```
TCT GTT ATT GAA AAG ATG AAC ACA CAA TTC ACA GCA GTG GGT AAA GAA 1248
Ser Val Ile Glu Lys Met Asn Thr Gln Phe Thr Ala Val Gly Lys Glu
                405                 410                 415

TTC AAC CAC CTG GAA AAA AGA ATA GAG AAT TTA AAC AAA AAG GTT GAT 1296
Phe Asn His Leu Glu Lys Arg Ile Glu Asn Leu Asn Lys Lys Val Asp
            420                 425                 430

GAT GGT TTT CTG GAT GTT TGG ACT TAC AAT GCC GAA CTG TTG GTT CTA 1344
Asp Gly Phe Leu Asp Val Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu
        435                 440                 445

TTG GAA AAT GAA AGA ACT TTG GAT TAT CAC GAT TCA AAT GTG AAG AAC 1392
Leu Glu Asn Glu Arg Thr Leu Asp Tyr His Asp Ser Asn Val Lys Asn
    450                 455                 460

CTA TAT GAG AAA GTA AGA AGC CAG CTA AAA AAC AAT GCC AAG GAA ATT 1440
Leu Tyr Glu Lys Val Arg Ser Gln Leu Lys Asn Asn Ala Lys Glu Ile
465                 470                 475                 480

GGA AAT GGC TGC TTT GAA TTT TAC CAC AAA TGT GAT GAC ACG TGC ATG 1488
Gly Asn Gly Cys Phe Glu Phe Tyr His Lys Cys Asp Asp Thr Cys Met
                485                 490                 495

GAG AGC GTC AAA AAT GGG ACT TAT GAT TAC CCA AAA TAC TCA GAG GAA 1536
Glu Ser Val Lys Asn Gly Thr Tyr Asp Tyr Pro Lys Tyr Ser Glu Glu
            500                 505                 510

GCA AAA CTA AAC AGA GAG GAG ATA GAT GGG GTA AAG CTG GAA TCA ACA 1584
Ala Lys Leu Asn Arg Glu Glu Ile Asp Gly Val Lys Leu Glu Ser Thr
        515                 520                 525

AGG ATT TAC CAG ATT TTG GCG ATC TAT TCA ACT GTC GCC AGT TCA TTG 1632
Arg Ile Tyr Gln Ile Leu Ala Ile Tyr Ser Thr Val Ala Ser Ser Leu
    530                 535                 540

GTA CTG TTA GTC TCC CTG GGG GCA ATC AGT TTC TGG ATG TGC TCC AAT 1680
Val Leu Leu Val Ser Leu Gly Ala Ile Ser Phe Trp Met Cys Ser Asn
545                 550                 555                 560

GGG TCT TTA CAG TGC AGA ATA TGT ATT TAA AAT TAG GAT CC 1721
Gly Ser Leu Gln Cys Arg Ile Cys Ile  .  Asn  .  Asp
                565                 570
```

(2) INFORMATION FOR SEQ ID NO:227:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 573 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:227:

```
Met Asn Ser Asp Pro Ala Ile Leu Leu Val Leu Leu Cys Thr Phe Thr
 1               5                  10                  15

Thr Ala Asn Ala Asp Thr Leu Cys Ile Gly Tyr His Ala Asn Asn Ser
            20                  25                  30

Thr Asp Thr Val Asp Thr Val Leu Glu Lys Asn Val Thr Val Thr His
        35                  40                  45

Ser Val Asn Leu Leu Glu Asp Arg His Asn Gly Lys Leu Cys Lys Leu
    50                  55                  60

Arg Gly Val Ala Pro Leu His Leu Gly Lys Cys Asn Ile Ala Gly Trp
65                  70                  75                  80

Leu Leu Gly Asn Pro Glu Cys Glu Leu Leu Phe Thr Ala Ser Ser Trp
                85                  90                  95

Ser Tyr Ile Val Glu Thr Ser Asn Ser Asp Asn Gly Thr Cys Tyr Pro
            100                 105                 110

Gly Asp Phe Ile Asn Tyr Glu Glu Leu Arg Glu Gln Leu Ser Ser Val
        115                 120                 125

Ser Ser Phe Glu Arg Phe Glu Ile Phe Pro Lys Ala Ser Ser Trp Pro
    130                 135                 140

Asn His Glu Thr Asn Ile Gly Val Thr Ala Ala Cys Pro Tyr Ala Gly
145                 150                 155                 160

Ala Asn Ser Phe Tyr Arg Asn Leu Ile Trp Leu Val Lys Lys Gly Asn
                165                 170                 175

Ser Tyr Pro Lys Leu Ser Lys Ser Tyr Ile Asn Asn Lys Glu Lys Glu
            180                 185                 190

Val Leu Val Leu Trp Gly Ile His His Pro Pro Thr Ser Thr Asp Gln
        195                 200                 205

Gln Ser Leu Tyr Gln Asn Ala Asp Ala Tyr Val Phe Val Gly Ser Ser
    210                 215                 220

Lys Tyr Asn Lys Lys Phe Lys Pro Glu Ile Ala Thr Arg Pro Lys Val
225                 230                 235                 240

Arg Gly Gln Ala Gly Arg Met Asn Tyr Tyr Trp Thr Leu Val Lys Pro
                245                 250                 255

Gly Asp Thr Ile Thr Phe Glu Ala Thr Gly Asn Leu Val Val Pro Arg
            260                 265                 270

Tyr Ala Phe Ala Met Lys Arg Gly Ser Gly Ser Gly Ile Ile Ile Ser
        275                 280                 285

Asp Thr Pro Val His Asp Cys Asn Thr Thr Cys Gln Thr Pro Lys Gly
    290                 295                 300

Ala Ile Asn Thr Ser Leu Pro Phe Gln Asn Ile His Pro Val Thr Ile
305                 310                 315                 320

Gly Glu Cys Pro Lys Tyr Val Lys Ser Thr Lys Leu Arg Met Ala Thr
                325                 330                 335

Gly Leu Arg Asn Ile Pro Ser Ile Gln Ser Arg Gly Leu Phe Gly Ala
            340                 345                 350

Ile Ala Gly Phe Ile Glu Gly Gly Trp Thr Gly Met Ile Asp Gly Trp
```

```
        355                 360                 365

Tyr Gly Tyr His His Gln Asn Glu Gln Gly Ser Gly Tyr Ala Ala Asp
    370                 375                 380

Arg Lys Ser Thr Gln Asn Ala Ile Asp Gly Ile Thr Asn Lys Val Asn
385                 390                 395                 400

Ser Val Ile Glu Lys Met Asn Thr Gln Phe Thr Ala Val Gly Lys Glu
                405                 410                 415

Phe Asn His Leu Glu Lys Arg Ile Glu Asn Leu Asn Lys Lys Val Asp
            420                 425                 430

Asp Gly Phe Leu Asp Val Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu
        435                 440                 445

Leu Glu Asn Glu Arg Thr Leu Asp Tyr His Asp Ser Asn Val Lys Asn
    450                 455                 460

Leu Tyr Glu Lys Val Arg Ser Gln Leu Lys Asn Asn Ala Lys Glu Ile
465                 470                 475                 480

Gly Asn Gly Cys Phe Glu Phe Tyr His Lys Cys Asp Asp Thr Cys Met
                485                 490                 495

Glu Ser Val Lys Asn Gly Thr Tyr Asp Tyr Pro Lys Tyr Ser Glu Glu
            500                 505                 510

Ala Lys Leu Asn Arg Glu Glu Ile Asp Gly Val Lys Leu Glu Ser Thr
        515                 520                 525

Arg Ile Tyr Gln Ile Leu Ala Ile Tyr Ser Thr Val Ala Ser Ser Leu
    530                 535                 540

Val Leu Leu Val Ser Leu Gly Ala Ile Ser Phe Trp Met Cys Ser Asn
545                 550                 555                 560

Gly Ser Leu Gln Cys Arg Ile Cys Ile  .  Asn  .  Asp
                565                 570
```

(2) INFORMATION FOR SEQ ID NO:228:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1414 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: N

(iv) ANTI-SENSE: N

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1414
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:228:

```
ATG AAT TCA AAT CAA AAA ATA ATA ACC ATT GGG TCA ATC TGT CTG ATA
48
Met Asn Ser Asn Gln Lys Ile Ile Thr Ile Gly Ser Ile Cys Leu Ile
  1               5                  10                  15
```

```
GTT GGA ATA GTT AGT CTA TTA TTG CAG ATA GGA AAT ATA GTC TCG TTA
96
Val Gly Ile Val Ser Leu Leu Leu Gln Ile Gly Asn Ile Val Ser Leu
            20                  25                  30

TGG ATA AGC CAT TCA ATT CAG ACT GGA GAA AAA AAC CAC TCT GAG ATA
144
Trp Ile Ser His Ser Ile Gln Thr Gly Glu Lys Asn His Ser Glu Ile
        35                  40                  45

TGC AAC CAA AAT ATC ATT ACA TAT GAA AAC AAC ACA TGG GTG AAC CAA
192
Cys Asn Gln Asn Ile Ile Thr Tyr Glu Asn Asn Thr Trp Val Asn Gln
    50                  55                  60

ACT TAT GTA AAC ATT AGC AAT ACC AAC ATT GCT GAT GGA CAG GGC GTG
240
Thr Tyr Val Asn Ile Ser Asn Thr Asn Ile Ala Asp Gly Gln Gly Val
65                  70                  75                  80

ACT TCA ATA ATA CTA GCC GGC AAT CCC CCT CTT TGC CCA ATA ATT GGG
288
Thr Ser Ile Ile Leu Ala Gly Asn Pro Pro Leu Cys Pro Ile Ile Gly
                85                  90                  95

TGG GCT ATA TAC AGC AAA AAC AAT AGC ATA AGG ATT GGT CCC AAA GGA
336
Trp Ala Ile Tyr Ser Lys Asn Asn Ser Ile Arg Ile Gly Pro Lys Gly
            100                 105                 110

AAC ATT TTT GTC ATA AAA AAA CCA TCC ATT TCA TGC TCT CAC TTG GAG
384
Asn Ile Phe Val Ile Lys Lys Pro Ser Ile Ser Cys Ser His Leu Glu
        115                 120                 125

TGC AAA ACC TTT TTC CTG ACC CAA GGT GCT TTG CTA AAT GAC AGG CAT
432
Cys Lys Thr Phe Phe Leu Thr Gln Gly Ala Leu Leu Asn Asp Arg His
    130                 135                 140

CCT AAT GGA ACC GTC AAG GAC AGG AGC CCT TAC CGA ACC TTA ATG AGC
480
Pro Asn Gly Thr Val Lys Asp Arg Ser Pro Tyr Arg Thr Leu Met Ser
145                 150                 155                 160

TGC CCG ATC GGT GAA GCT CCA TCT CCG TAT AAT TCA AGA TTC GAA TCA
528
Cys Pro Ile Gly Glu Ala Pro Ser Pro Tyr Asn Ser Arg Phe Glu Ser
                165                 170                 175

GTT GCT TGG TCA GCA AGT GCA TGC CAT GAT GGA ATG GGA TGG CTA ACA
576
Val Ala Trp Ser Ala Ser Ala Cys His Asp Gly Met Gly Trp Leu Thr
            180                 185                 190

ATC GGG ATT TCC GGT CCA GAT AAT GGA GCA GTG GCT GTT TTG AAA TAC
624
Ile Gly Ile Ser Gly Pro Asp Asn Gly Ala Val Ala Val Leu Lys Tyr
        195                 200                 205

AAT GGT ATA ATA ACA GAT ACA ATA AAA AGT TGG AGA AAC AAA ATA CTA
672
Asn Gly Ile Ile Thr Asp Thr Ile Lys Ser Trp Arg Asn Lys Ile Leu
    210                 215                 220

AGA ACA CAA GAG TCA GAA TGT GTT TGT ATA AAC GGT TCA TGT TTT ACT
720
```

Arg Thr Gln Glu Ser Glu Cys Val Cys Ile Asn Gly Ser Cys Phe Thr
225 230 235 240

ATA ATG ACT GAT GGC CCA AGC AAT GGG CAA GCC TCG TAC AAA ATA TTC
768
Ile Met Thr Asp Gly Pro Ser Asn Gly Gln Ala Ser Tyr Lys Ile Phe
245 250 255

AAA ATG GAG AAA GGG AAG ATT ATT AAG TCA GTT GAG CTG GAT GCA CCT
816
Lys Met Glu Lys Gly Lys Ile Ile Lys Ser Val Glu Leu Asp Ala Pro
260 265 270

AAT TAC CAC TAT GAG GAA TGC TCC TGT TAC CCT GAT ACA GGC AAA GTG
864
Asn Tyr His Tyr Glu Glu Cys Ser Cys Tyr Pro Asp Thr Gly Lys Val
275 280 285

GTG TGT GTG TGC AGA GAC AAT TGG CAT GCT TCA AAT CGA CCG TGG GTC
912
Val Cys Val Cys Arg Asp Asn Trp His Ala Ser Asn Arg Pro Trp Val
290 295 300

TCT TTC GAT CAG AAT CTT GAT TAT CAG ATA GGG TAC ATA TGC AGT GGG
960
Ser Phe Asp Gln Asn Leu Asp Tyr Gln Ile Gly Tyr Ile Cys Ser Gly
305 310 315 320

GTT TTC GGT GAT AAT CCG CGT TCT AAT GAT GGG AAA GGC AAT TGT GGC
1008
Val Phe Gly Asp Asn Pro Arg Ser Asn Asp Gly Lys Gly Asn Cys Gly
325 330 335

CCA GTA CTT TCT AAT GGA GCA AAT GGA GTG AAA GGA TTC TCA TTT AGA
1056
Pro Val Leu Ser Asn Gly Ala Asn Gly Val Lys Gly Phe Ser Phe Arg
340 345 350

TAT GGC AAT GGT GTT TGG ATA GGA AGA ACT AAA AGT ATC AGC TCT AGA
1104
Tyr Gly Asn Gly Val Trp Ile Gly Arg Thr Lys Ser Ile Ser Ser Arg
355 360 365

AGT GGA TTT GAG ATG ATT TGG GAT CCA AAT GGA TGG ACG GAA ACT GAT
1152
Ser Gly Phe Glu Met Ile Trp Asp Pro Asn Gly Trp Thr Glu Thr Asp
370 375 380

AGT AGT TTC TCT ATA AAG CAG GAT ATT ATA GCA TTA ACT GAT TGG TCA
1200
Ser Ser Phe Ser Ile Lys Gln Asp Ile Ile Ala Leu Thr Asp Trp Ser
385 390 395 400

GGA TAC AGT GGA AGT TTT GTC CAA CAT CCT GAA TTA ACA GGA ATG AAC
1248
Gly Tyr Ser Gly Ser Phe Val Gln His Pro Glu Leu Thr Gly Met Asn
405 410 415

TGC ATA AGG CCT TGT TTT TGG GTA GAG TTA ATC AGA GGA CAA CCC AAG
1296
Cys Ile Arg Pro Cys Phe Trp Val Glu Leu Ile Arg Gly Gln Pro Lys
420 425 430

GAG AGC ACA ATC TGG ACT AGT GGA AGC AGC ATT TCT TTC TGT GGC GTG
1344
Glu Ser Thr Ile Trp Thr Ser Gly Ser Ser Ile Ser Phe Cys Gly Val
435 440 445

```
GAC AAT GAA ACC GCA AGC TGG TCA TGG CCA GAC GGA GCT GAT CTG CCA
1392
Asp Asn Glu Thr Ala Ser Trp Ser Trp Pro Asp Gly Ala Asp Leu Pro
    450                 455                     460

TTC ACC ATT GAC AAG TAG ATC T
1414
Phe Thr Ile Asp Lys  .  Ile
465                 470
```

(2) INFORMATION FOR SEQ ID NO:229:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 471 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:229:

```
Met Asn Ser Asn Gln Lys Ile Ile Thr Ile Gly Ser Ile Cys Leu Ile
  1               5                  10                  15

Val Gly Ile Val Ser Leu Leu Leu Gln Ile Gly Asn Ile Val Ser Leu
             20                  25                  30

Trp Ile Ser His Ser Ile Gln Thr Gly Glu Lys Asn His Ser Glu Ile
         35                  40                  45

Cys Asn Gln Asn Ile Ile Thr Tyr Glu Asn Asn Thr Trp Val Asn Gln
     50                  55                  60

Thr Tyr Val Asn Ile Ser Asn Thr Asn Ile Ala Asp Gly Gln Gly Val
 65                  70                  75                  80

Thr Ser Ile Ile Leu Ala Gly Asn Pro Pro Leu Cys Pro Ile Ile Gly
                 85                  90                  95

Trp Ala Ile Tyr Ser Lys Asn Asn Ser Ile Arg Ile Gly Pro Lys Gly
            100                 105                 110

Asn Ile Phe Val Ile Lys Lys Pro Ser Ile Ser Cys Ser His Leu Glu
        115                 120                 125

Cys Lys Thr Phe Phe Leu Thr Gln Gly Ala Leu Leu Asn Asp Arg His
    130                 135                 140

Pro Asn Gly Thr Val Lys Asp Arg Ser Pro Tyr Arg Thr Leu Met Ser
145                 150                 155                 160

Cys Pro Ile Gly Glu Ala Pro Ser Pro Tyr Asn Ser Arg Phe Glu Ser
                165                 170                 175

Val Ala Trp Ser Ala Ser Ala Cys His Asp Gly Met Gly Trp Leu Thr
            180                 185                 190

Ile Gly Ile Ser Gly Pro Asp Asn Gly Ala Val Ala Val Leu Lys Tyr
        195                 200                 205

Asn Gly Ile Ile Thr Asp Thr Ile Lys Ser Trp Arg Asn Lys Ile Leu
    210                 215                 220

Arg Thr Gln Glu Ser Glu Cys Val Cys Ile Asn Gly Ser Cys Phe Thr
225                 230                 235                 240
```

```
Ile Met Thr Asp Gly Pro Ser Asn Gly Gln Ala Ser Tyr Lys Ile Phe
                245                 250                 255

Lys Met Glu Lys Gly Lys Ile Ile Lys Ser Val Glu Leu Asp Ala Pro
            260                 265                 270

Asn Tyr His Tyr Glu Glu Cys Ser Cys Tyr Pro Asp Thr Gly Lys Val
        275                 280                 285

Val Cys Val Cys Arg Asp Asn Trp His Ala Ser Asn Arg Pro Trp Val
    290                 295                 300

Ser Phe Asp Gln Asn Leu Asp Tyr Gln Ile Gly Tyr Ile Cys Ser Gly
305                 310                 315                 320

Val Phe Gly Asp Asn Pro Arg Ser Asn Asp Gly Lys Gly Asn Cys Gly
                325                 330                 335

Pro Val Leu Ser Asn Gly Ala Asn Gly Val Lys Gly Phe Ser Phe Arg
            340                 345                 350

Tyr Gly Asn Gly Val Trp Ile Gly Arg Thr Lys Ser Ile Ser Ser Arg
        355                 360                 365

Ser Gly Phe Glu Met Ile Trp Asp Pro Asn Gly Trp Thr Glu Thr Asp
    370                 375                 380

Ser Ser Phe Ser Ile Lys Gln Asp Ile Ile Ala Leu Thr Asp Trp Ser
385                 390                 395                 400

Gly Tyr Ser Gly Ser Phe Val Gln His Pro Glu Leu Thr Gly Met Asn
                405                 410                 415

Cys Ile Arg Pro Cys Phe Trp Val Glu Leu Ile Arg Gly Gln Pro Lys
            420                 425                 430

Glu Ser Thr Ile Trp Thr Ser Gly Ser Ser Ile Ser Phe Cys Gly Val
        435                 440                 445

Asp Asn Glu Thr Ala Ser Trp Ser Trp Pro Asp Gly Ala Asp Leu Pro
    450                 455                 460

Phe Thr Ile Asp Lys     Ile
465                 470
```

(2) INFORMATION FOR SEQ ID NO:230:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1501 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: N

(iv) ANTI-SENSE: N

(ix) FEATURE:
- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1501
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:230:

ATG AAT TCT CAA GGC ACC AAA CGA TCA TAT GAA CAA ATG GAG ACT GGT
48
Met Asn Ser Gln Gly Thr Lys Arg Ser Tyr Glu Gln Met Glu Thr Gly
1 5 10 15

GGG GAA CGC CAG GAT GCC ACA GAA ATC AGA GCA TCT GTC GGA AGA ATG
96
Gly Glu Arg Gln Asp Ala Thr Glu Ile Arg Ala Ser Val Gly Arg Met
20 25 30

ATT GGT GGA ATC GGA AGA TTC TAC ATC CAA ATG TGC ACT GAA CTC AAA
144
Ile Gly Gly Ile Gly Arg Phe Tyr Ile Gln Met Cys Thr Glu Leu Lys
35 40 45

CTC AGT GAC TAT GAG GGA CGA CTA ATT CAA AAT AGC ATA ACA ATA GAG
192
Leu Ser Asp Tyr Glu Gly Arg Leu Ile Gln Asn Ser Ile Thr Ile Glu
50 55 60

AGA ATG GTG CTC TCT GCT TTT GAT GAG AGA AGG AAT AAA TAC CTA GAA
240
Arg Met Val Leu Ser Ala Phe Asp Glu Arg Arg Asn Lys Tyr Leu Glu
65 70 75 80

GAG CAT CCC AGT GCT GGG AAG GAT CCT AAG AAA ACT GGA GGA CCC ATA
288
Glu His Pro Ser Ala Gly Lys Asp Pro Lys Lys Thr Gly Gly Pro Ile
85 90 95

TAT AGA AGG GTA GAC GGA AAA TGG ATG AGA GAA CTC ATC CTT TAT GAC
336
Tyr Arg Arg Val Asp Gly Lys Trp Met Arg Glu Leu Ile Leu Tyr Asp
100 105 110

AAA GAA GAA ATA AGG AGA GTT TGG CGC CAA GCA AAC AAT GGT GAG GAT
384
Lys Glu Glu Ile Arg Arg Val Trp Arg Gln Ala Asn Asn Gly Glu Asp
115 120 125

GCA ACA GCC GGT CTT ACT CAC ATC ATG ATT TGG CAC TCC AAT CTT AAT
432
Ala Thr Ala Gly Leu Thr His Ile Met Ile Trp His Ser Asn Leu Asn
130 135 140

GAT GCC ACC TAT CAG AGA ACA AGA GCG CTT GTT CGC ACT GGA ATG GAT
480
Asp Ala Thr Tyr Gln Arg Thr Arg Ala Leu Val Arg Thr Gly Met Asp
145 150 155 160

CCC AGA ATG TGC TCC CTA ATG CAA GGT TCA ACA CTT CCC AGA AGG TCT
528
Pro Arg Met Cys Ser Leu Met Gln Gly Ser Thr Leu Pro Arg Arg Ser
165 170 175

GGG GCC GCA GGT GCT GCA GTG AAA GGA GTT GGA ACA ATA GCA ATG GAG
576
Gly Ala Ala Gly Ala Ala Val Lys Gly Val Gly Thr Ile Ala Met Glu
180 185 190

TTA ATC AGA ATG ATC AAA CGT GGA ATC AAT GAC CGA AAC TTC TGG AGG
624
Leu Ile Arg Met Ile Lys Arg Gly Ile Asn Asp Arg Asn Phe Trp Arg
195 200 205

GGT GAA AAT GGA CGA AGG ACA AGG ATT GCA TAT GAA AGA ATG TGC AAT
672

Gly Glu Asn Gly Arg Arg Thr Arg Ile Ala Tyr Glu Arg Met Cys Asn
210 215 220

ATT CTC AAA GGA AAA TTT CAG ACA GCT GCC CAG AGG GCA ATG ATG GAT 720
Ile Leu Lys Gly Lys Phe Gln Thr Ala Ala Gln Arg Ala Met Met Asp
225 230 235 240

CAA GTA AGA GAA AGT CGA AAC CCA GGA AAC GCT GAA ATT GAA GAT CTC 768
Gln Val Arg Glu Ser Arg Asn Pro Gly Asn Ala Glu Ile Glu Asp Leu
245 250 255

ATT TTC CTG GCA CGG TCA GCA CTT ATT CTA AGG GGG TCA GTT GCA CAT 816
Ile Phe Leu Ala Arg Ser Ala Leu Ile Leu Arg Gly Ser Val Ala His
260 265 270

AAG TCC TGC CTG CCT GCT TGT GTG TAT GGG CTT GCA GTA GCA AGT GGG 864
Lys Ser Cys Leu Pro Ala Cys Val Tyr Gly Leu Ala Val Ala Ser Gly
275 280 285

CAT GAC TTT GAA AGA GAA GGA TAT TCA CTG GTC GGG ATA GAC CCC TTC 912
His Asp Phe Glu Arg Glu Gly Tyr Ser Leu Val Gly Ile Asp Pro Phe
290 295 300

AAA TTA CTT CAA AAC AGT CAA GTG TTC AGC CTG ATC AGA CCA AAT GAA 960
Lys Leu Leu Gln Asn Ser Gln Val Phe Ser Leu Ile Arg Pro Asn Glu
305 310 315 320

AAC CCA GCT CAC AAG AGT CAA TTG GTG TGG ATG GCA TGC CAT TCT GCT 1008
Asn Pro Ala His Lys Ser Gln Leu Val Trp Met Ala Cys His Ser Ala
325 330 335

GCA TTT GAG GAT TTA AGA ATA TCA AGT TTC ATA AGA GGG AAG AAA GTG 1056
Ala Phe Glu Asp Leu Arg Ile Ser Ser Phe Ile Arg Gly Lys Lys Val
340 345 350

GTT CCA AGA GGA AAG CTT TCC ACA AGA GGG GTT CAG ATT GCT TCA AAT 1104
Val Pro Arg Gly Lys Leu Ser Thr Arg Gly Val Gln Ile Ala Ser Asn
355 360 365

GAG AAT GTG GAA GCT ATG GAC TCT AGT ACC CTA AAA CTA AGA AGC AGA 1152
Glu Asn Val Glu Ala Met Asp Ser Ser Thr Leu Lys Leu Arg Ser Arg
370 375 380

TAT TGG GCC ATA AGG ACC AGA AGT GGA GGA AAT ACC AAC CAA CAG AAG 1200
Tyr Trp Ala Ile Arg Thr Arg Ser Gly Gly Asn Thr Asn Gln Gln Lys
385 390 395 400

GCA TCT GCG GGC CAG ATC AGT GTG CAA CCT ACA TTC TCA GTG CAA CGG 1248
Ala Ser Ala Gly Gln Ile Ser Val Gln Pro Thr Phe Ser Val Gln Arg
405 410 415

AAT CTC CCT TTT GAA AGA GCA ACC GTT ATG GCA GCT TTC AGC GGG AAT 1296
Asn Leu Pro Phe Glu Arg Ala Thr Val Met Ala Ala Phe Ser Gly Asn
420 425 430

AAT GAG GGA CGG ACA TCA GAC ATG CGA ACG GAA GTT ATA AGG ATG ATG
1344
Asn Glu Gly Arg Thr Ser Asp Met Arg Thr Glu Val Ile Arg Met Met
435 440 445

GAA AGT GCA AAG CCA GAA GAT TTG TCC TTC CAG GGG CGG GGA GTC TTC
1392
Glu Ser Ala Lys Pro Glu Asp Leu Ser Phe Gln Gly Arg Gly Val Phe
450 455 460

GAG CTC TCG GAC GAA AAG GCA ACG AAC CCG ATC GTG CCT TCC TTT GAC
1440
Glu Leu Ser Asp Glu Lys Ala Thr Asn Pro Ile Val Pro Ser Phe Asp
465 470 475 480

ATG AGT AAT GAA GGG TCT TAT TTC TTC GGA GAC AAT GCA GAG GAG TAT
1488
Met Ser Asn Glu Gly Ser Tyr Phe Phe Gly Asp Asn Ala Glu Glu Tyr
485 490 495

GAC AAT TGA ATT C
1501
Asp Asn . Ile
500

(2) INFORMATION FOR SEQ ID NO:231:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 500 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:231:

Met Asn Ser Gln Gly Thr Lys Arg Ser Tyr Glu Gln Met Glu Thr Gly
1 5 10 15

Gly Glu Arg Gln Asp Ala Thr Glu Ile Arg Ala Ser Val Gly Arg Met
20 25 30

Ile Gly Gly Ile Gly Arg Phe Tyr Ile Gln Met Cys Thr Glu Leu Lys
35 40 45

Leu Ser Asp Tyr Glu Gly Arg Leu Ile Gln Asn Ser Ile Thr Ile Glu
50 55 60

Arg Met Val Leu Ser Ala Phe Asp Glu Arg Arg Asn Lys Tyr Leu Glu
65 70 75 80

Glu His Pro Ser Ala Gly Lys Asp Pro Lys Lys Thr Gly Gly Pro Ile
85 90 95

Tyr Arg Arg Val Asp Gly Lys Trp Met Arg Glu Leu Ile Leu Tyr Asp
100 105 110

Lys Glu Glu Ile Arg Arg Val Trp Arg Gln Ala Asn Asn Gly Glu Asp
115 120 125

Ala Thr Ala Gly Leu Thr His Ile Met Ile Trp His Ser Asn Leu Asn
130 135 140

Asp Ala Thr Tyr Gln Arg Thr Arg Ala Leu Val Arg Thr Gly Met Asp
145 150 155 160

```
Pro Arg Met Cys Ser Leu Met Gln Gly Ser Thr Leu Pro Arg Arg Ser
                165                 170                 175

Gly Ala Ala Gly Ala Ala Val Lys Gly Val Gly Thr Ile Ala Met Glu
            180                 185                 190

Leu Ile Arg Met Ile Lys Arg Gly Ile Asn Asp Arg Asn Phe Trp Arg
        195                 200                 205

Gly Glu Asn Gly Arg Arg Thr Arg Ile Ala Tyr Glu Arg Met Cys Asn
    210                 215                 220

Ile Leu Lys Gly Lys Phe Gln Thr Ala Ala Gln Arg Ala Met Met Asp
225                 230                 235                     240

Gln Val Arg Glu Ser Arg Asn Pro Gly Asn Ala Glu Ile Glu Asp Leu
                245                 250                 255

Ile Phe Leu Ala Arg Ser Ala Leu Ile Leu Arg Gly Ser Val Ala His
            260                 265                 270

Lys Ser Cys Leu Pro Ala Cys Val Tyr Gly Leu Ala Val Ala Ser Gly
        275                 280                 285

His Asp Phe Glu Arg Glu Gly Tyr Ser Leu Val Gly Ile Asp Pro Phe
    290                 295                 300

Lys Leu Leu Gln Asn Ser Gln Val Phe Ser Leu Ile Arg Pro Asn Glu
305                 310                 315                     320

Asn Pro Ala His Lys Ser Gln Leu Val Trp Met Ala Cys His Ser Ala
                325                 330                 335

Ala Phe Glu Asp Leu Arg Ile Ser Ser Phe Ile Arg Gly Lys Lys Val
            340                 345                 350

Val Pro Arg Gly Lys Leu Ser Thr Arg Gly Val Gln Ile Ala Ser Asn
        355                 360                 365

Glu Asn Val Glu Ala Met Asp Ser Ser Thr Leu Lys Leu Arg Ser Arg
    370                 375                 380

Tyr Trp Ala Ile Arg Thr Arg Ser Gly Gly Asn Thr Asn Gln Gln Lys
385                 390                 395                     400

Ala Ser Ala Gly Gln Ile Ser Val Gln Pro Thr Phe Ser Val Gln Arg
                405                 410                 415

Asn Leu Pro Phe Glu Arg Ala Thr Val Met Ala Ala Phe Ser Gly Asn
            420                 425                 430

Asn Glu Gly Arg Thr Ser Asp Met Arg Thr Glu Val Ile Arg Met Met
        435                 440                 445

Glu Ser Ala Lys Pro Glu Asp Leu Ser Phe Gln Gly Arg Gly Val Phe
    450                 455                 460

Glu Leu Ser Asp Glu Lys Ala Thr Asn Pro Ile Val Pro Ser Phe Asp
465                 470                 475                     480

Met Ser Asn Glu Gly Ser Tyr Phe Phe Gly Asp Asn Ala Glu Glu Tyr
                485                 490                 495

Asp Asn  .  Ile
            500
```

<u>What is claimed is:</u>

1. A recombinant swinepox virus comprising a foreign DNA inserted into a swinepox virus genome, wherein the foreign DNA is inserted into an EcoRI site within a region corresponding to a 3.2 Kb subfragment of the HindIII K fragment which contains both a HindIII and an EcoRI site, of the swinepox virus genome and is capable of being expressed in a host cell into which the virus is introduced.

2. A recombinant swinepox virus comprising a foreign DNA inserted into a swinepox virus genome, wherein the foreign DNA is inserted into a) an AccI site within a region corresponding to a 3.6 Kb HindIII to BglII subfragment of the HindIII M fragment and b) an EcoRI site within a region corresponding to a 3.2 Kb subfragment of the HindIII K fragment which contains both a HindIII and EcoRI site, of the swinepox virus genome and is capable of being expressed in a host cell into which the virus is introduced.

3. The recombinant swinepox virus of claim 1, wherein the foreign DNA encodes a polypeptide.

4. The recombinant swinepox virus of claim 1, wherein the foreign DNA encodes E. coli beta-galactosidase or beta-glucuronidase.

5. The recombinant swinepox virus of claim 1, wherein the foreign DNA is under control of a heterologous upstream promoter.

6. The recombinant swinepox virus of claim 5, wherein the promoter is: synthetic pox viral promoter, pox synthetic late promoter 1, pox early promoter 2, pox synthetic late promoter 2 early promoter 2, pox synthetic early promoter 2, pox O1L promoter, pox I4L promoter, pox I3L promoter, pox I2L promoter, pox I1L promoter, pox E10R promoter, PRV gX, HSV-1 alpha 4, internal ribosomal entry site, and HCMV immediate early.

7. The recombinant swinepox virus of claim 1, wherein the polypeptide is: swine influenza virus hemagglutinin, swine influenza virus neurominidase, swine influenza virus matrix and swine influenza virus nucleoprotein.

8. The recombinant swinepox virus of claim 7, which is designated S-SPV-120.

9. The recombinant swinepox virus of claim 7, which is designated S-SPV-121.

10. The recombinant swinepox virus of claim 7, which is designated S-SPV-122.

11. The recombinant swinepox virus of claim 1, wherein the foreign DNA encodes a cytokine.

12. The recombinant swinepox virus of claim 1, wherein the polypeptide is: swine influenza

virus hemagglutinin, swine influenza virus neurominidase, swine influenza virus matrix, swine influenza virus nucleoprotein, pseudorabies virus virus glycoprotein B, pseudorabies virus glycoprotein C, pseudorabies virus glycoprotein D, porcine respiratory and reproductive virus ORF2, porcine respiratory and reproductive virus ORF3, porcine respiratory and reproductive virus ORF4, porcine respiratory and reproductive virus ORF5, porcine respiratory, and reproductive virus ORF6, and porcine respiratory and reproductive virus ORF7.

13. The recombinant swinepox virus of claim 12, which is designated S-SPV-131.

14. The recombinant swinepox virus of claim 12, which is designated S-SPV-132.

15. The recombinant swinepox virus of claim 1, wherein the polypeptide is: feline leukemia virus surface protein, feline leukemia virus transmembrane protein, feline leukemia virus gag, feline leukemia virus transmembrane protease, feline immunodeficiency virus gag/protease, feline immunodeficiency virus envelope, feline leukemia virus gag/protease, feline leukemia virus envelope, canine parvovirus VP2, and canine parvovirus VP1/2.

16. The recombinant swinepox virus of claim 15, which is designated S-SPV-127.

17. The recombinant swinepox virus of claim 15, which is designated S-SPV-128.

18. The recombinant swinepox virus of claim 15, which is designated S-SPV-205.

19. The recombinant swinepox virus of claim 15, which is designated S-SPV-206.

20. The recombinant swinepox virus of claim 15, which is designated S-SPV-207.

21. The recombinant swinepox virus of claim 1, wherein the polypeptide is: bovine cytokine interleukin-12 protein 35, bovine cytokine interleukin-12 protein 40, Bovine Respiratory Syncytial Virus glycoprotein G, Newcastles Disease fusion, Infectious Rhinotracheitis Virus glycoprotein D, Canine Distemper Virus fusion, Canine Distemper Virus Hemagluttinin, DV HA, Bovine Viral Diarrhea Virus type 1 glycoprotein 45, Bovine Viral Diarrhea Virus type 1 glycoprotein 48, Bovine Viral Diarrhea Virus type 1 glycoprotein 53, Bovine Viral Diarrhea Virus type 2 glycoprotein 53.

22. The recombinant swinepox virus of claim 21, which is designated S-SPV-142.

23. The recombinant swinepox virus of claim 21, which is designated S-SPV-143.

24. The recombinant swinepox virus of claim 21, which is designated S-SPV-176.

25. The recombinant swinepox virus of claim 21, which is designated S-SPV-184.

26. The recombinant swinepox virus of claim 21, which is designated S-SPV-185.

27. The recombinant swinepox virus of claim 21, which is designated S-SPV-188.

28. The recombinant swinepox virus of claim 21, which is designated S-SPV-202.

29. A recombinant swinepox virus designated S-SPV-164.

30. A recombinant swinepox virus designated S-SPV-183.

31. A recombinant swinepox virus designated S-SPV-198.

32. A recombinant swinepox virus designated S-SPV-200.

33. A recombinant swinepox virus designated S-SPV-217.

34. A vaccine useful for immunizing an animal against swinepox virus which comprises an effective immunizing amount of the recombinant swinepox virus of claims 1 and a suitable carrier.

35. A method of immunizing an animal against a pathogen which comprises administering to the

animal an effective immunizing dose of the vaccine of claim 34.

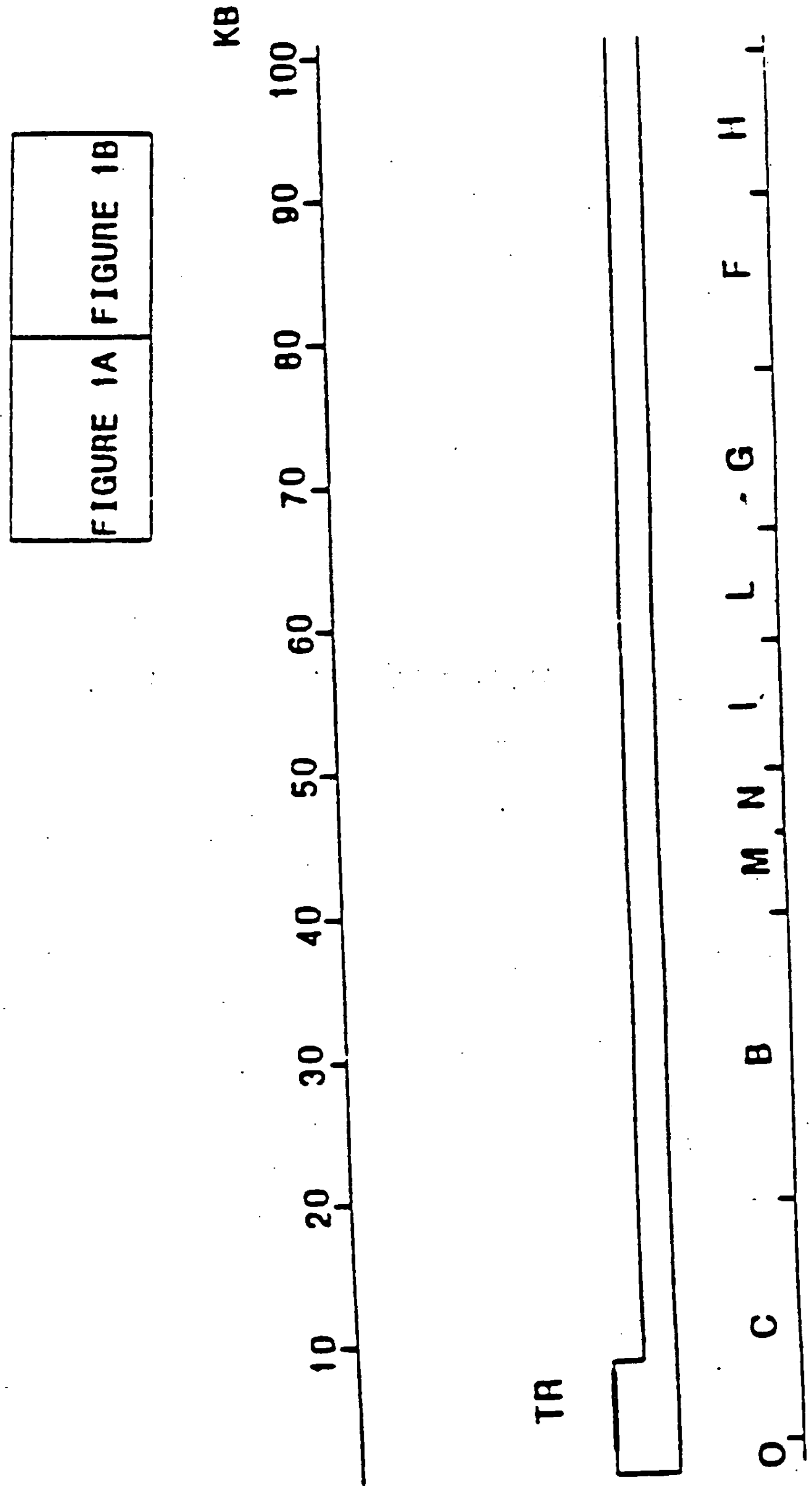


FIGURE 1A
FIGURE 1B
KB
100
90
80
70
60
50
40
30
20
10
TR
O
C
B
M
N
I
L
G
F
H

FIGURE 1A

THIS PAGE BLANK (USPTO)

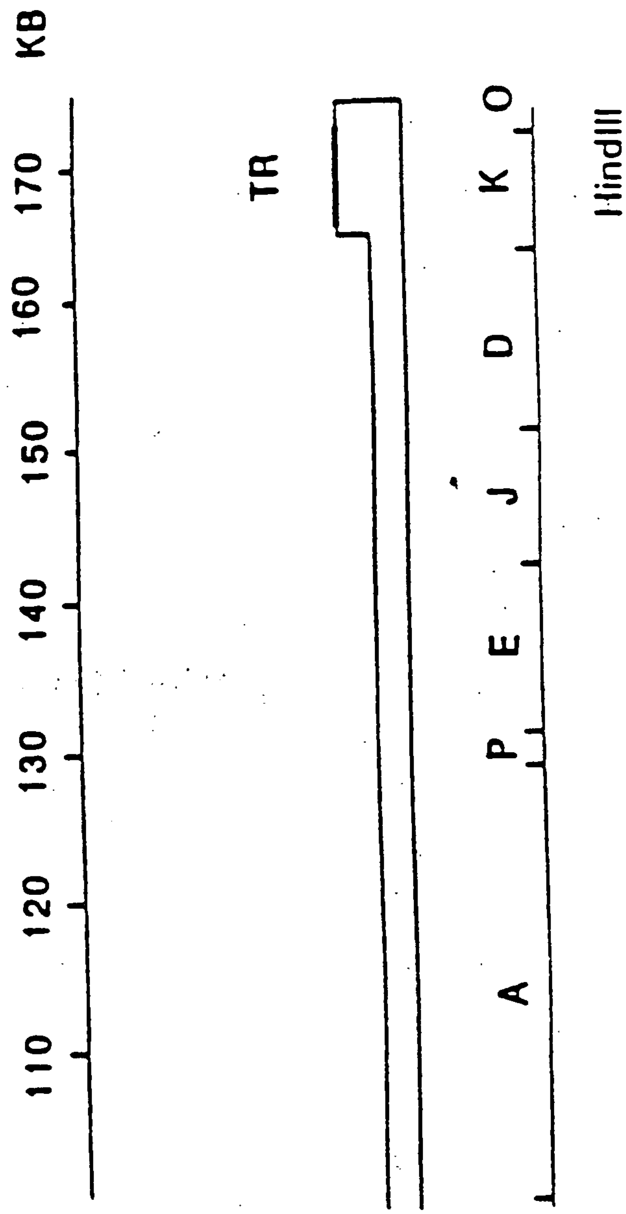
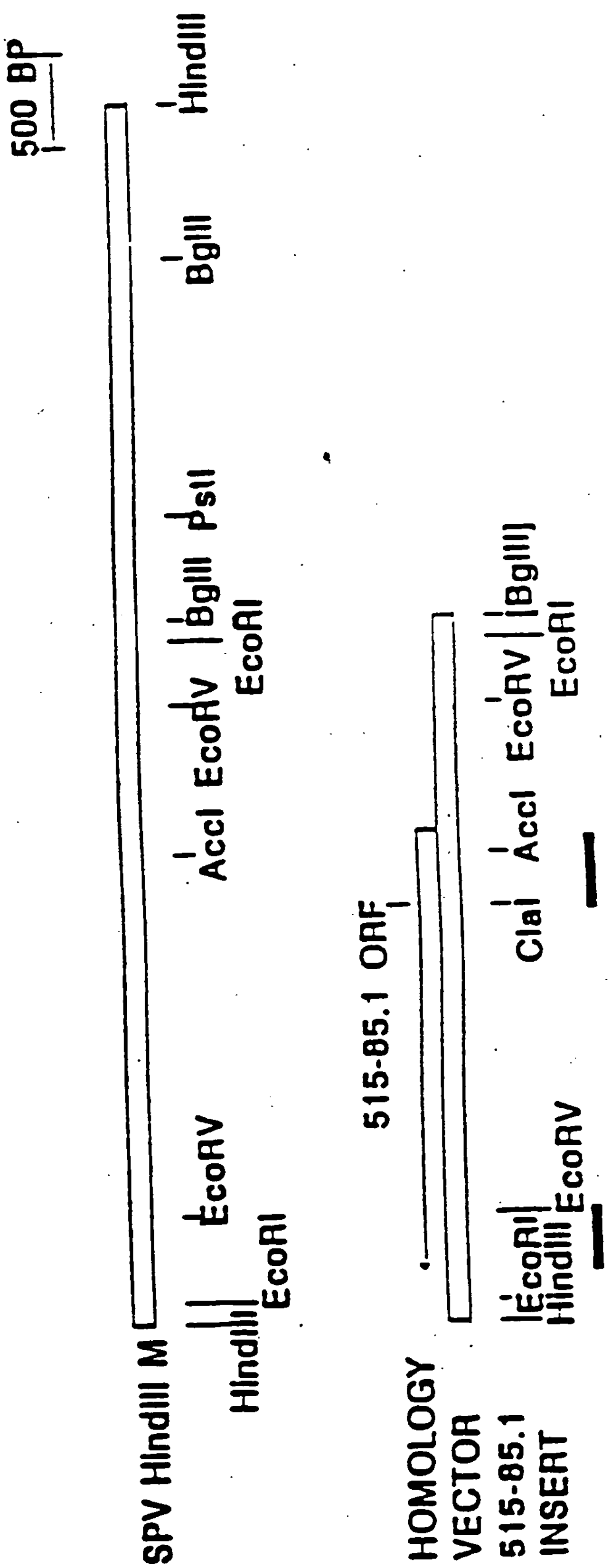


FIGURE 1B

THIS PAGE BLANK (USPTO)

FIGURE 2A


500 BP
SPV HindIII M
HindIII
EcoRI
EcoRV
AccI
EcoRV
BglII
EcoRI
PstI
BglII
HindIII
515-85.1 ORF
HOMOLOGY
VECTOR
515-85.1
INSERT
EcoRI
HindIII
EcoRV
ClaI
AccI
EcoRV
EcoRI
[BglII]

THIS PAGE BLANK (USPTO)

FIGURE 2B

```
                 10        20        30        40        50        60        70
                  *         *         *         *         *         *         *
(A) VV       MFMYPEFARKALSKLISKKLNIEKVSSKHQLVLLDYGLHGLLPKSLYLEAINSDILNVRFFPPEIINVT
orf 01L        :::  :::  :: :: : : :    :     | | :: ::   :  :  :: : :: :     :
(B) SPV     MPSYMYPKNARKVISKIISLQLDIKKLPKKYINTMLEFGLHGNLPACMYKDAVSYDINNIRFLPYNCVMVK
AccI-ClaI                                        |
                                                AccI

                 80        90        100       110       120
                  *         *         *         *         *
(A) VV       DIVKALQNSCRVDEYLKAVSLYHKNSLMVSGPNVVK-LMIEYNLLTHSDLEWLINENVVKA
orf 01L      :       :  :  :      :  :   :      :::  ::   :
(B) SPV      DLINVIKSSSVIDTRLHQSVLKHRRALIDYGDQDIITLMIINKLLSIDDISYILDKKIIHV
AccI-ClaI                                             |
                                                     ClaI
```

THIS PAGE BLANK (USPTO)

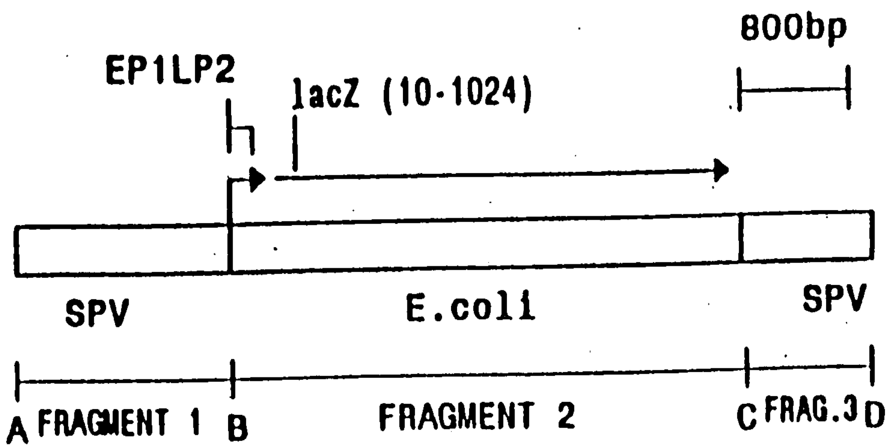
FIGURE 2C

```
                        570       580       590       600       610
(C) VV orf O1L          VLNDQYAKIVIFFNTIIEYIIATIYYRLTVLNNYTNVKIIFVSKVLHTVMEA
(D) SPV EcoRV-EcoRI     SLNEYYSKIVILINVILEYMISIILYRILIVKRFNNIKEFISKVVNTVLES
                                              EcoRV

                        620       630       640       650       660         TERM
(C) VV orf O1L          CGVLFSYIKVNDKIEHELEEMVDKGTVPSYLYHLSINVISIILDDINGTR-
(D) SPV EcoRV-EcoRI     SGIYFCQMRVHEQIELEIDELIINGSMPVQLMHLLLKVATIILEEIKEI-    ——▶ EcoRI
                                                                         TERM
```

THIS PAGE BLANK (USPTO)

FIGURE 3A



DNA	ORIGIN	SITES	SIZE
VECTOR	pSP64	HindIII-BamHI	~2972 BP
FRAGMENT 1	SPV HindIII M	HindIII-AccI	~2149 BP
FRAGMENT 2	pJF751	BamHI-PvuII	~3002 BP
FRAGMENT 3	SPV HindIII M	AccI-BglII	~1484 BP

THIS PAGE BLANK (USPTO)

FIGURE 3B

HindIII

JUNCTION A CAC ATA CGA TTT AGG=TGA CAC TAT AGA ATA=CAA GCT TTA TAC CAT=TAT AGA

→ SPV HindIII M

pSP64 ←

JUNCTION A (CONTINUED)

TAC ATT ACC=TTG TCC GAC GTG TAG=AAT TCA TGC CAA AGA=AGA ATT AAC TAA

[AccI] XbaI EP1

JUNCTION B GTA GTC=GAC TCT AGA AAA AAT=TGA AAA ACT ATT CTA=ATT TAT TGC ACG G

SPV HindIII M ←

EcoRI BamHI

JUNCTION B (CONTINUED)

BglII LP2

AG=ATC TTT TTT TTT TTT=TTT TTT TTT GGC ATA=TAA ATG AAT TCG GAT=CCC GTC

MET Asn Ser Asp Pro Val

→ lacZ(10)

→ pJF751

THIS PAGE BLANK (USPTO)

FIGURE 3C

PvuII

JUNCTION C AGC CCG TCA GTA=TCG GCG GAA ATC CAG=CTG AGC GCC GGT CGC=TAC CAT TAC CAG
Ser Pro Ser Val Ser Ala Glu Ile Gln Leu Ser Ala Gly Arg Tyr His Tyr Gln

pJF751

JUNCTION C (CONTINUED)

SmaI [AccI]

TTG=GTC TGG TGT CAA AAA=GAT CCA TAA TTA ATT=AAC CCG GGT CGA AGA=C
Leu Val Trp Cys Gln Lys Asp Pro ---

lacZ (1024)

SPV HindIII M

[BglII]
[BamHI] SmaI SacI EcoRI

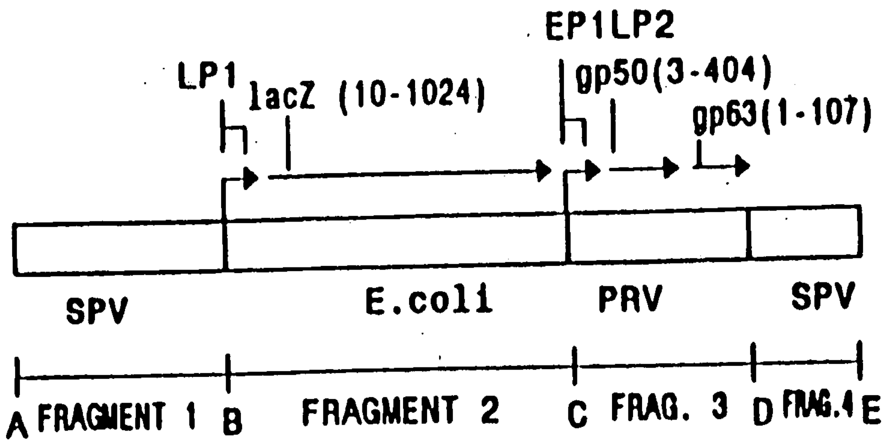
JUNCTION D AGA TCC CCG GGC GAG=CTC GAA TTC GTA ATC=ATG GTC ATA GTT=TCC

pSP64

SPV HindIII M

THIS PAGE BLANK (USPTO)

FIGURE 4A



DNA	ORIGIN	SITES	SIZE
VECTOR	pSP64	HindIII-BamHI	~2972 BP
FRAGMENT 1	SPV HindIII M	HindIII-AccI	~2149 BP
FRAGMENT 2	pJF751	BamHI-PvuII	~3002 BP
FRAGMENT 3	PRV BamHI #7	EcoRI*-StuI	~1558 BP
FRAGMENT 4	SPV HindIII M	AccI-BglII	~1484 BP

*INTRODUCED VIA CLONING

THIS PAGE BLANK (USPTO)

FIGURE 4B

```
                                             HindIII
JUNCTION A  CAC ATA CGA TTT AGG=TGA CAC TAT AGA ATA=CAA GCT TTA TAC CAT=TAT AGA TAC ATT
                                        pSP64 <----|
                                              |----------> SPV HindIII M

                            [AccI]           PstI   SalI   XbaI
JUNCTION B  AAG CTG GTA GAT TTC=CAT GTA GGG CCG CCT=GCA GGT CGA CTC TAG=A

               SPV HindIII M <-----|

JUNCTION B (CONTINUED)
                     LP1                           EcoRI   BamHI
  AT TTC ATT TTG TTT=TTT TCT ATG CTA TAA=ATG AAT TCG GAT CCC=GTC GTT TTA CAA
                                         MET Asn Ser Asp Pro Val Val Leu Gln
                                                         |----> lacZ(10)
                                                 |-------------> pJF751
```

THIS PAGE BLANK (USPTO)

FIGURE 4C

```
                     PvuII
JUNCTION C  GTA TCG GCG GAA ATC CAG CTG AGC=GCC GGT CGC TAC CAT=TAC CAG TTG GTC TGG=TGT
            Val Ser Ala Glu Ile Gln Leu Ser Ala Gly Arg Tyr His Tyr Gln Leu Val Trp Cys
               pJF751

                                                 PstI   SalI
CAA=AAA GAT CCA TAA TTA=ATT AAC CCG GCC GCC=TGC AGG TGG ACT
Gln Lys Asp Pro ---

lacZ (1024)

XbaI              EP1                                  BglII
CT AGA AAA AAT TGA=AAA ACT ATT CTA ATT=TAT TGC ACG GAG ATC=T

                LP2                     EcoRI
TT TTT TTT TTT TTT=TTT TTT GGC ATA TAA=ATG AAT TCG CTC GCA=GCG CTA TTG GCG GCG
                                       MET Asn Ser Leu Ala Ala Leu Leu Ala Ala
                                                   gp50(3)
                                               PRV BamHI #7
```

THIS PAGE BLANK (USPTO)

FIGURE 4D

[StuI] XbaI BamHI

JUNCTION D CGC GTG CAC CAC GAG=GGA CTC TAG AGG ATC=CAT AAT TAA TTA ATT=AA

Arg Val His His Glu

gp63(107)

PRV BamHI #7

JUNCTION D (CONTINUED)

ETTS SmaI SalI PstI SalI PstI [AccI]

T TTT TAT CCC GGG=TCG ACC TGC AGG CGG=CCG GGT CGA CCT GCA=GGC GGC CAG AC

SPV HindIII M

[BglII],[BamHI] SacI EcoRI

JUNCTION E AGA TCC CCG GGC GAG=CTC GAA TTC GTA ATC=ATG GTC ATA GCT GTT=TCC TGT GTG AAA

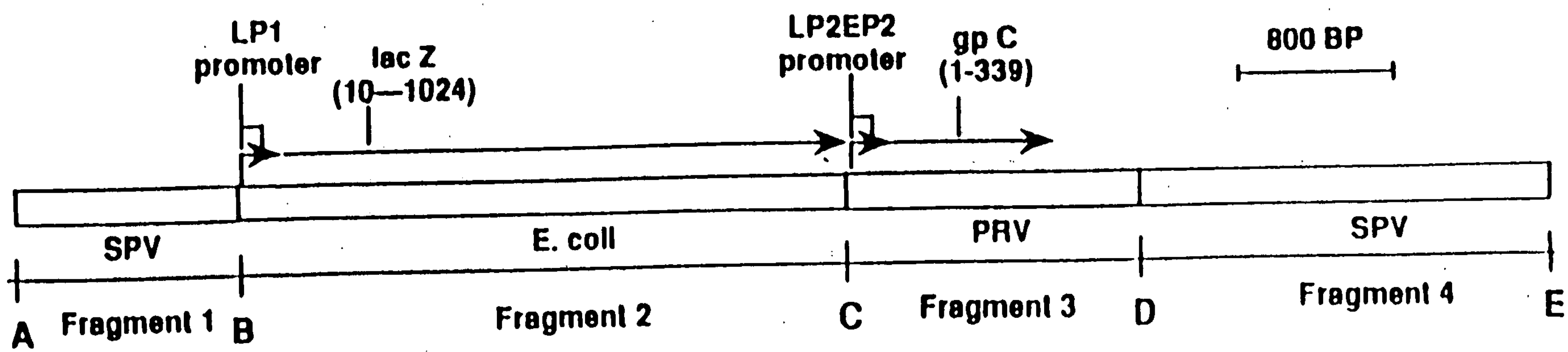
SPV HindIII M

pSP64

THIS PAGE BLANK (USPTO)

FIGURE 5A

DNA	Origin	Sites	Size
Vector	pSP64	Hind III—Bam HI	~2972 BP
Fragment 1	SPV HindIII M	Bgl II—Acc I	~1484 BP
Fragment 2	pJF751	Bam HI—Pvu II	~3002 BP
Fragment 3	PRV BamHI 2 & 9	Nco I—Nco I	~2378 BP
Fragment 4	SPV HindIII M	Acc I—Hind III	~ 2149 BP



THIS PAGE BLANK (USPTO)

FIGURE 5B

JUNCTION A

[Bam HI/Bgl II]

ACA GGA AAC AGC TAT GAC CAT GAT TAC GAA TTC GAG CTC GCC CGG GGA TCT

SPV Hind III M

pSP64

JUNCTION B

[Acc I] LP1

AA ATA TAT AAA TAC CAT GTT AGA ATT TGG TCT GCT GCA GGT CGA CTC TAG AAT TTC ATT TTG

SPV Hind III M

JUNCTION B (CONT.)

LP1 Eco R I Bam H I

TTT TTT TCT ATG CTA TAA ATG AAT TCG GAT CCC GTC GTT TTA

MET Asn Ser Asp Pro Val Val Leu

E. coli Lac Z (10)

pJF 751

THIS PAGE BLANK (USPTO)

FIGURE 5C

JUNCTION C

```
                Pvu II
GAA ATC CAG CTG AGC GCC GGT CGC TAC CAT TAC CAG TTG GTC TGG TGT CAA AAA GAT
Glu Il  Gln Leu Ser Ala Gly Arg Tyr His Tyr Gln Leu Val Trp Cys Gln Lys Asp
        <-- pJF 751                                   <-- E. coli Lac Z (1024)
```

JUNCTION C (CONT.)

```
                        Sal I   Xba I                LP2
CCA TAA TTA ATT AAC CCG GTC GAC TCT AGA TTT TTT TTT TTT TTT TTT TTG GCA TAT AAA
Pro  •
```

JUNCTION C (CONT.)

```
   Bgl II                         EP2                           Eco R I    [Nco I]
TAG ATC TGT ATC CTA AAA TTG AAT TGT AAT TAT CGA TAA TAA ATG AAT TCC GGC ATG GCC TCG
                                                         MET Asn Ser Gly Met Ala Ser
                                                                         --> PRV gpC (1)
```

THIS PAGE BLANK (USPTO)

FIGURE 5D

JUNCTION D

[Nco I]

C CAT GCT CTA GAG GAT CCC CGG GCG AGC TCG AAT TCG GAT CCA TAA TTA ATT

PRV Bam HI #9

EcoRI

JUNCTION D (CONT.)

[Acc I]

AAT TAA TTT TTA TCC CGG GTC GAC CGG GTC GAC CTG CAG CCT ACA TGG AAA TCT ACC

SPV Hind III M

JUNCTION E

HindIII

TAA TGT ATC TAT AAT GGT ATA AAG CTT GTA TTC TAT AGT GTC ACC TAA ATC

pSP64

SPV Hind III M

THIS PAGE BLANK (USPTO)

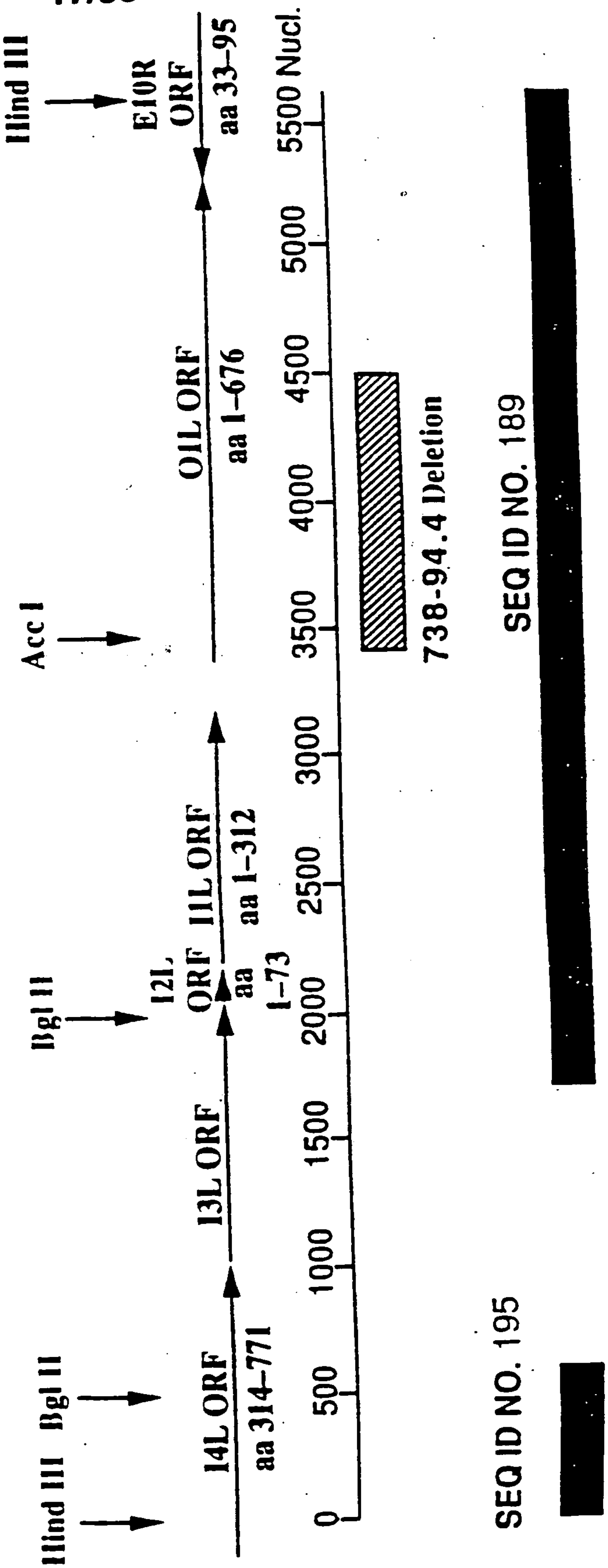
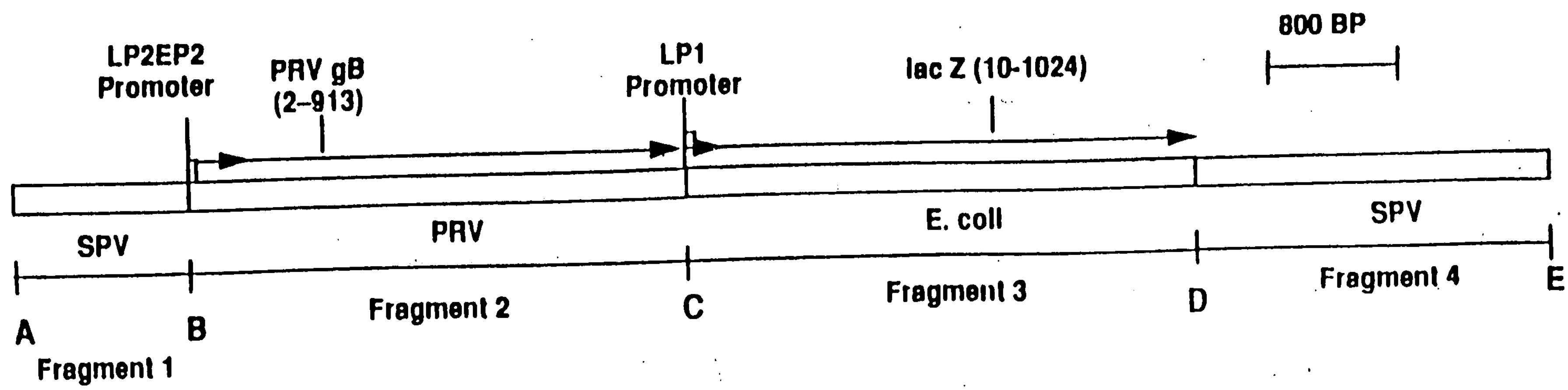

Hind III
Bgl II
Bgl II
Acc I
Hind III
I4L ORF
aa 314-771
I3L ORF
I2L ORF
aa 1-73
I1L ORF
aa 1-312
O1L ORF
aa 1-676
E10R ORF
aa 33-95
0
500
1000
1500
2000
2500
3000
3500
4000
4500
5000
5500 Nucl.
738-94.4 Deletion
SEQ ID NO. 195
SEQ ID NO. 189

FIGURE 6

THIS PAGE BLANK (USPTO)

FIGURE 7A

DNA	Origin	Sites	Size
Vector	pSP64	Hind III–Bam HI	~2972 BP
Fragment 1	SPV Hind III M	Bgl II–Acc I	~1484 BP
Fragment 2	PRV Kpn I C	Sma I–Sac I	~3500 BP
Fragment 3	pJF751	Bam HI–Pvu II	~3010 BP
Fragment 4	SPV Hind III M	Acc I–Hind III	~2149 BP



THIS PAGE BLANK (USPTO)

FIGURE 7B

Junction A

[Bam HI/Bgl II]

ACA GGA AAC AGC TAT GAC CAT GAT TAC GAA TTC GAG CTC GCC CGG GGA TCT

SPV Hind III M

pSP64

Junction B

[Acc I] Not I Sal I Xba I LP2

GTA TTGC GGC CGC CTG CAG GTC GAC TCT AGA TTT TTT TTT TTT TTT TTT TTG GCA TAT AAA

SPV Hind III M

Junction B Continued

Bgl II EP2

TAG ATC TGT ATC CTA AAA TTG AAT TGT AAT TAT CGA TAA TAA

Junction B Continu d

EcoR I Sma I

ATGAA TTC ACC CGC TGG TGG CGG TCT TTG GCG CGG GCC CCG TGG GCA TCG GCC CGG GCA CCA CGG

Synthetic N-terminal

PRV gB (2-16) (Synthetic)

PRV Kpn I C

THIS PAGE BLANK (USPTO)

FIGURE 7C

Junction C

Sac I, EcoR I, Bam H I, Asc I, LP1

GAG CTC GAAT TCG GAT CCA TAA TTA ATT AAT TTT TAT CCC GGC GCG CCT

PRV Kpn I C

Junction C Continued

LP1, EcoR I, Bam H I

CGA CTC TAG AAT TTC ATT TTG TTT TTT TCT ATG CTA TAA ATG AAT TCG GAT CCC GTC GTT TTA

E. coli Lac Z (10)

pJF 751

THIS PAGE BLANK (USPTO)

FIGURE 7D

Junction D

Pvu II

GAA ATC CAG CTG AGC GCC GGT CGC TAC CAT TAC CAG TTG GTC TGG TGT CAA AAA GAT

pJF 751

E. coli Lac Z (1024)

Junction D Continued

Asc I Sal I Not I [Acc I]

CCA TAA TTA ATT AAC CCG GGT CGA GGC GCG CCG GGT CGA CCT GCA GGC GGC CGC TAT AC

SPV Hind III M

Junction E

HindIII

TAA TGT ATC TAT AAT GGT ATA AAG CTT GTA TTC TAT AGT GTC ACC TAA ATC

pSP64

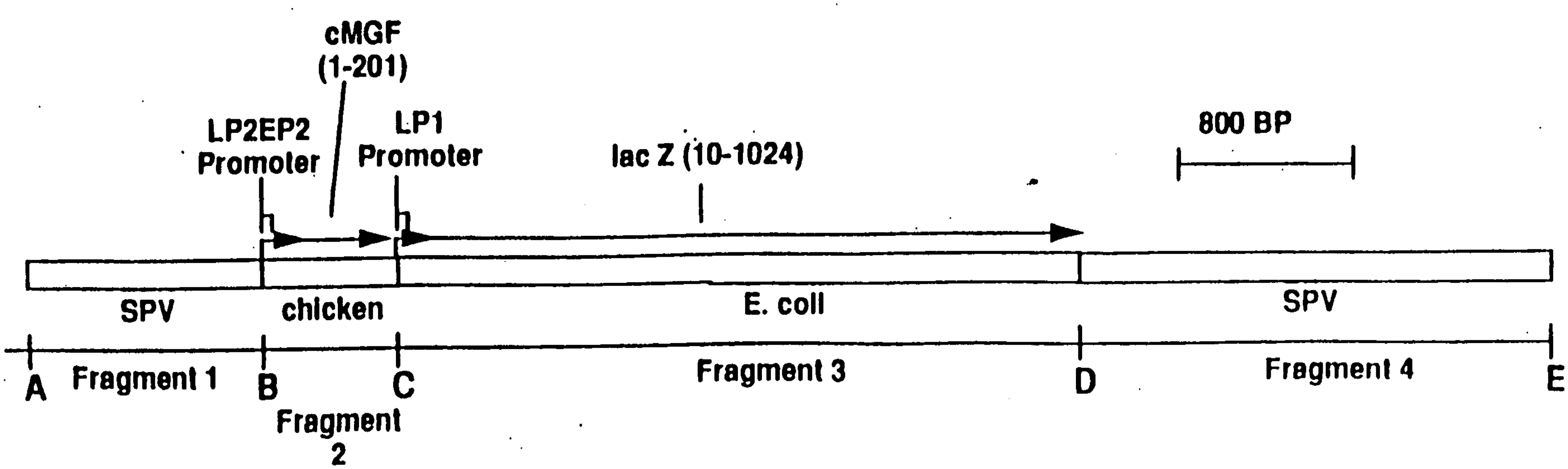
SPV Hind III M

THIS PAGE BLANK (USPTO)

FIGURE 8A

DNA	Origin	Sites	Size
Vector	pSP64	Hind III–Bam HI	~2972 BP
Fragment 1	SPV Hind III M	Bgl II–Acc I	~1484 BP
Fragment 2	chicken MGF	EcoR I†–BamH I†	~640 BP
Fragment 3	pJF751	Bam HI–Pvu II	~3002 BP
Fragment 4	SPV Hind III M	Acc I–Hind III	~2149 BP

†Restriction sites introduced by PCR cloning



THIS PAGE BLANK (USPTO)

FIGURE 8B

Junction A

[Bam HI/Bgl II]

ACA GGA AAC AGC TAT GAC CAT GAT TAC GAA TTC GAG CTC GCC CGG GGA TCT

SPV Hind III M

pSP64

Junction B

[Acc I] Not I Sal I Xba I LP2

GTAT AGC GGC CGC CTG CAG GTC GAC TCT AGA TTT TTT TTT TTT TTT TTT TTG GCA TAT AAA

SPV Hind III M

Junction B Continued

Bgl II EP2

TAG ATC TGT ATC CTA AAA TTG AAT TGT AAT TAT CGA TAA TAA AT

Junction B Continued

EcoRI

GAA TTC CAT GTG CTG CCT CAC CCC TGT GCT GGC GCT

cMGF (aa 1-201)

THIS PAGE BLANK (USPTO)

FIGURE 8C

Junction C

BamH I — Asc I — LP1

TCG CCC GCC TCT GAC GCC CCG GAT CCA TAA TTA ATT AAT TTT TAT CCC GGC GCG CCT

cMGF (aa 1-201)

Junction C Continued

LP1 — EcoR I — Bam H I

CGA CTC TAG AAT TTC ATT TTG TTT TTT TCT ATG CTA TAA ATG AAT TCG GAT CCC GTC GTT TTA

E. coli Lac Z (10)

pJF 751

THIS PAGE BLANK (USPTO)

FIGURE 8D

Junction D

Pvu II

GAA ATC CAG CTG AGC GCC GGT CGC TAC CAT TAC CAG TTG GTC TGG TGT CAA AAA GAT

pJF 751

E. coli Lac Z (1024)

Junction D Continued

Asc I — Sal I — Not I — [Acc I]

CCA TAA TTA ATT AAC CCG GGT CGA GGC GCG CCG GGT CGA CCT GCA GGC GGC CGC TAT AC

SPV Hind III M

Junction E

HindIII

TAA TGT ATC TAT AAT GGT ATA AAG CTT GTA TTC TAT AGT GTC ACC TAA ATC

pSP64

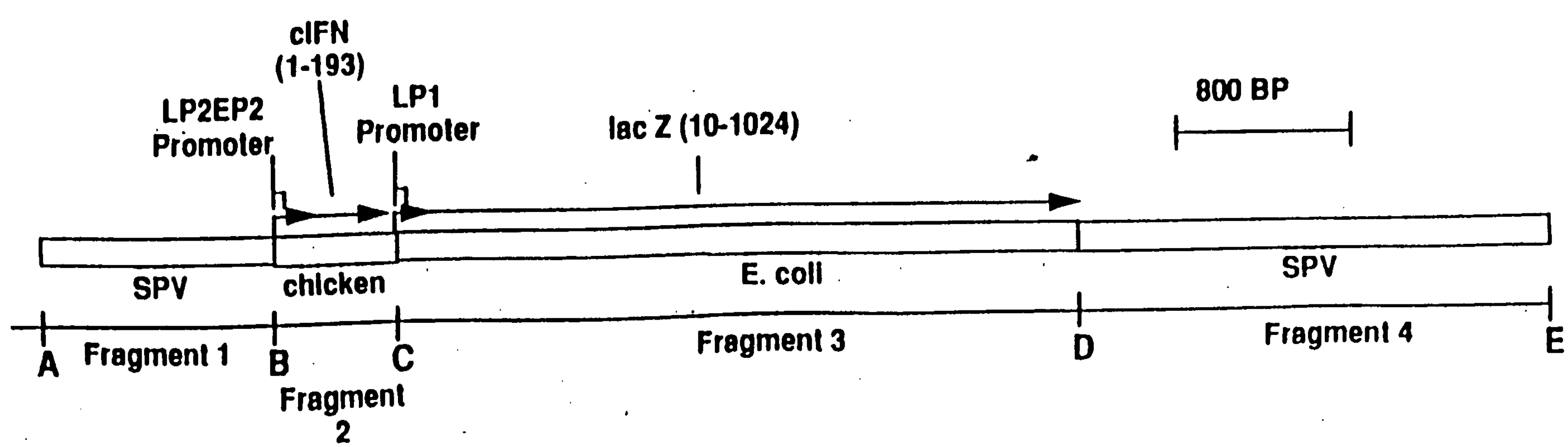
SPV Hind III M

THIS PAGE BLANK (USPTO)

FIGURE 9A

DNA	Origin	Sites	Size
Vector	pSP64	Hind III–Bam HI	~2972 BP
Fragment 1	SPV Hind III M	Bgl II–Acc I	~1484 BP
Fragment 2	chicken IFN	EcoR I†–Bgl II†	~577 BP
Fragment 3	pJF751	Bam HI–Pvu II	~3002 BP
Fragment 4	SPV Hind III M	Acc I–Hind III	~2149 BP

†Restriction sites introduced by PCR cloning



THIS PAGE BLANK (USPTO)

FIGURE 9B

Junction A

[Bam HI/Bgl II]

ACA GGA AAC AGC TAT GAC CAT GAT TAC GAA TTC GAG CTC GCC CGG GGA TCT

SPV Hind III M

pSP64

Junction B

[Acc I] Not I Sal I Xba I LP2

GTAT AGC GGC CGC CTG CAG GTC GAC TCT AGA TTT TTT TTT TTT TTT TTT TTG GCA TAT AAA

SPV Hind III M

Junction B Continued

Bgl II EP2

TAG ATC TGT ATC CTA AAA TTG AAT TGT AAT TAT CGA TAA TAA AT

Junction B Continued

EcoRI

GAA TTC GAT GGC TGT GCC TGC AAG CCC ACA GCA

cIFN (aa 1-193)

THIS PAGE BLANK (USPTO)

FIGURE 9C

Junction C

[Bgl II, Bam H I] Asc I LP1

CTT AGC CCC AAA CGC ACC TCA GAT CCA TAA TTA ATT AAT TTT TAT CCC GGC GCG CC

cIFN (aa 1-193)

Junction C Continued

LP1 EcoR I Bam H I

CGA CTC TAG AAT TTC ATT TTG TTT TTT TCT ATG CTA TAA ATG AAT TCG GAT CCC GTC GTT TTA

E. coli Lac Z (10)

pJF 751

THIS PAGE BLANK (USPTO)

FIGURE 9D

Junction D

Pvu II

GAA ATC CAG CTG AGC GCC GGT CGC TAC CAT TAC CAG TTG GTC TGG TGT CAA AAA GAT

pJF 751

E. coli Lac Z (1024)

Junction D Continued

Asc I Sal I Not I [Acc I]

CCA TAA TTA ATT AAC CCG GGT CGA GGC GCG CCG GGT CGA CCT GCA GGC GGC CGC TAT AC

SPV Hind III M

Junction E

HindIII

TAA TGT ATC TAT AAT GGT ATA AAG CTT GTA TTC TAT AGT GTC ACC TAA ATC

pSP64

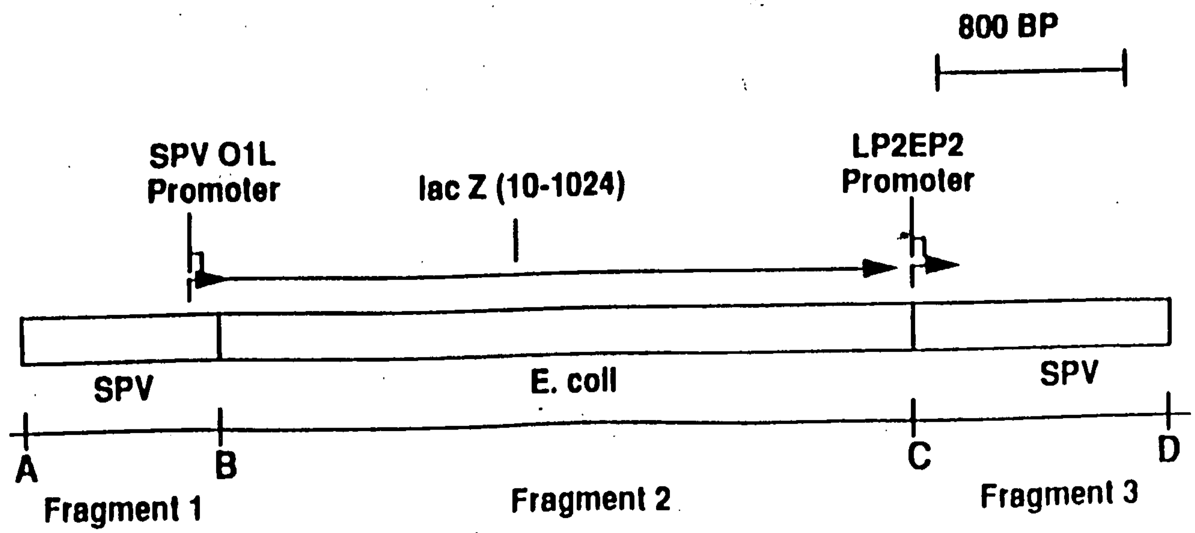
SPV Hind III M

THIS PAGE BLANK (USPTO)

FIGURE 10A

DNA	Origin	Sites	Size
Vector	pSP65	Hind III–Sph I	~2519 BP
Fragment 1	SPV Hind III M	Sph I–Bgl II†	~855 BP
Fragment 2	pJF751	Bam HI–Pvu II	~3002 BP
Fragment 3	SPV Hind III M	Sal I†–Hind III	~1113 BP

†Restriction sites introduced by PCR cloning



THIS PAGE BLANK (USPTO)

FIGURE 10B

Junction A

Sph I

CAA GGA ATG GTG CAT GCC CGT TCT TAT CAA TAG TTT AGT CGA AAA

pSP 65 ◄—|—► **SPV Hind III M**

Junction B

O1L Translation Start

[Bgl II, BamH I]

TAT ATA AGC ACT TAT TTT TGT TAG TAT AAT AAC ACA ATG CCA GAT CCC GTC GTT TTA

SPV Hind III M ◄—|—► ***E. coli* lac Z**

THIS PAGE BLANK (USPTO)

FIGURE 10C

Junction C

Pvu II

TCC AGC TGA GCG CCG GTC GCT ACC ATT AGC AGT TGG TCT GGT GTC AAA AAG ATC CAT AAT

pJF 751

E. coli lacZ (1024)

Junction C
Continued

Sal I Xba I LP2

TAA TTA ACC AGC GGC CGC CTG CAG GTC GAC TCT AGA TTT TTT TTT TTT TTT TTT TTG GCA

Junction C
Continu d

Bgl II EP2 EcoRI BamHI

TAT AAA TAG ATC TGT ATC CTA AAA TTG AAT TGT AAT TAT CGA TAA TAA ATG AAT TCG GAT CCA

Junction C
Continu d

Sal I Pst I Not I Sal I

TAA TTA ATT AAT TTT TAT CCC GGC GCG CCG GGT CGA CCT GCA GGC GGC CGC TGG GTC GAC AAA GAT

SPV Hind III M

THIS PAGE BLANK (USPTO)

FIGURE 10D

Junction D

Hind III

CAA AAG TCG TAA ATA CTG TAC TAG AAG CTT GGC GTA ATC ATG GTC

SPV Hind III M ←

→ pSP 65

THIS PAGE BLANK (USPTO)

FIGURE 11A

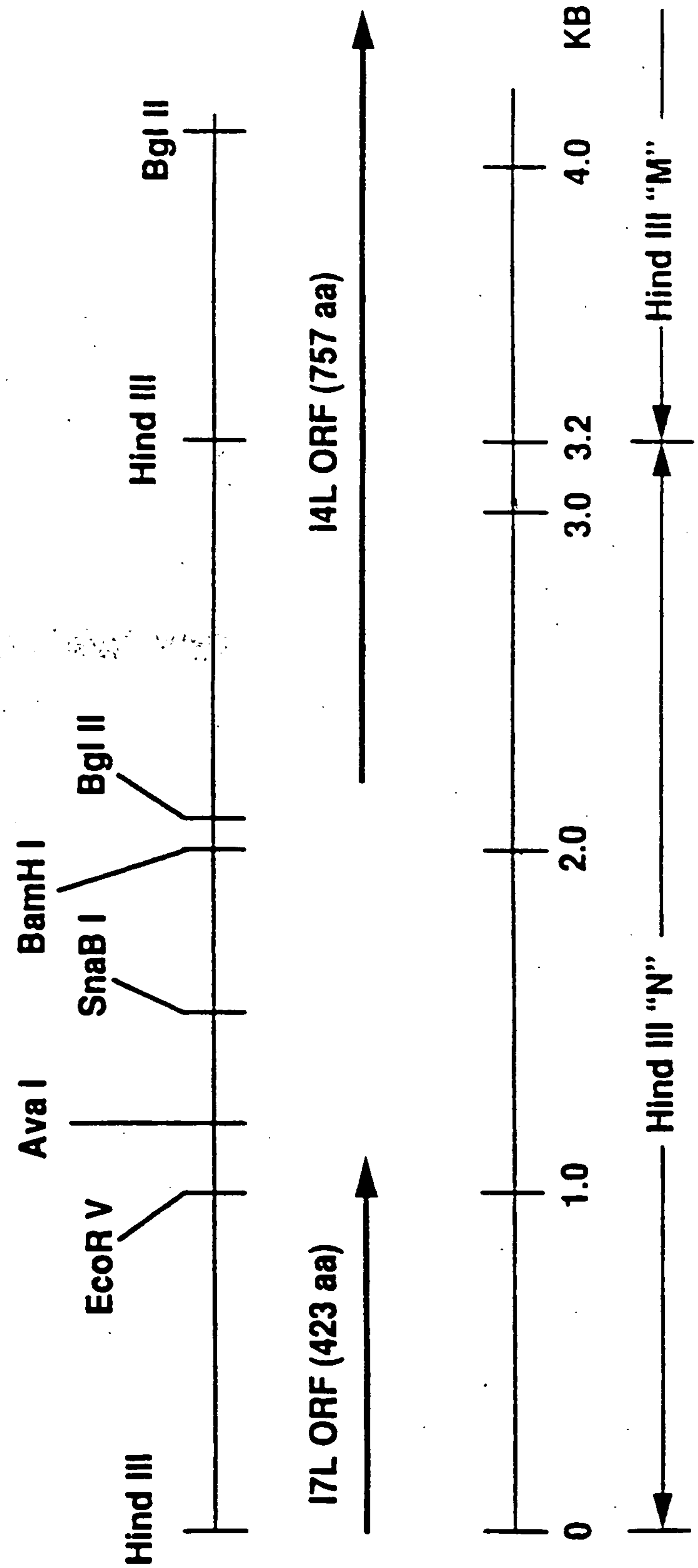
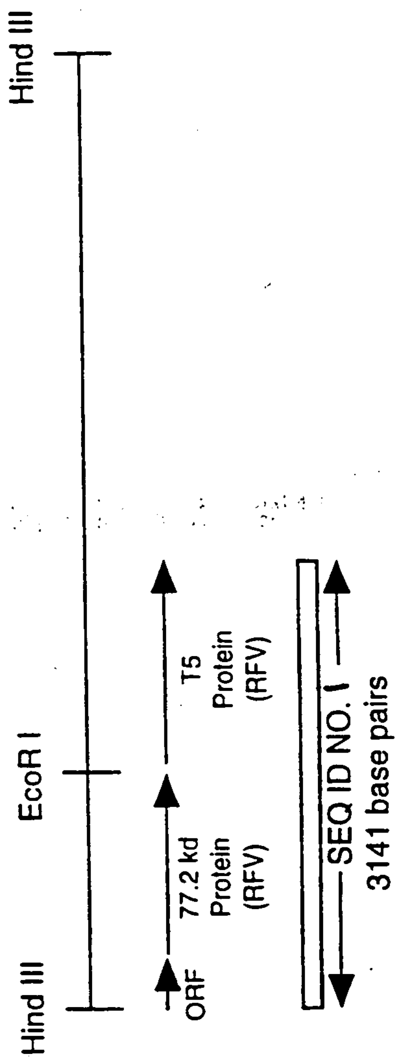

Hind III
EcoR V
Ava I
SnaB I
BamH I
Bgl II
Hind III
Bgl II
I7L ORF (423 aa)
I4L ORF (757 aa)
0
1.0
2.0
3.0
3.2
4.0
KB
Hind III "N"
Hind III "M"

FIGURE 11B

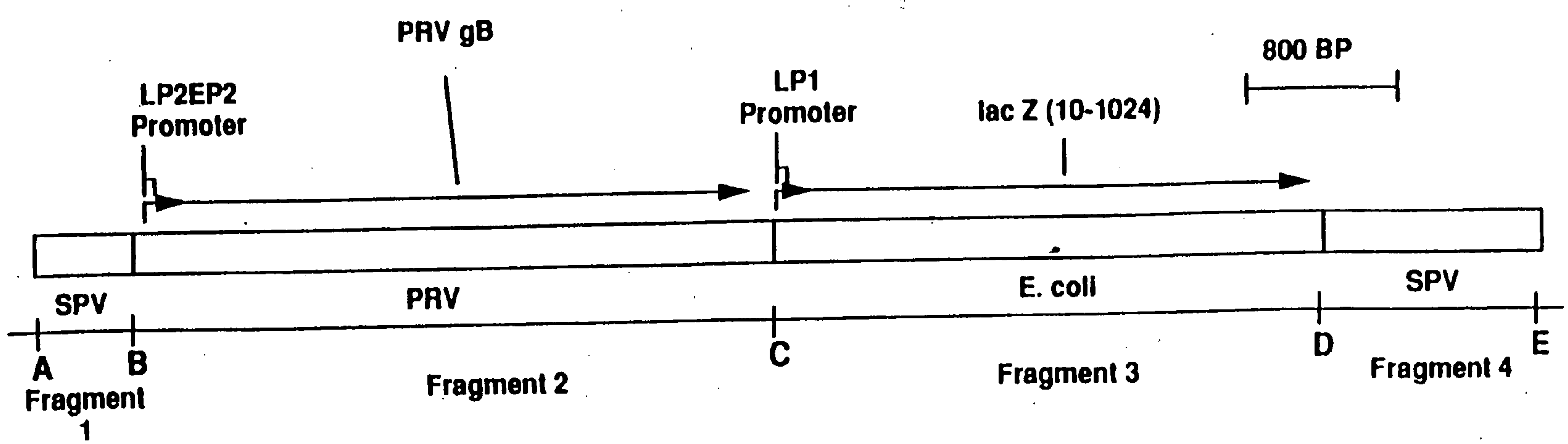
SPV HindIII K Genomic Fragment
6.7 kb



THIS PAGE BLANK (USPTO)

FIGURE 12A

DNA	Origin	Sites	Size
Vector	pSP64	Pst I–Hind III	~2986 BP
Fragment 1	SPV Hind III M	Hind III–Bgl II	~542 BP
Fragment 2	PRV Kpn I C	Sma I–Sac I	~3500 BP
Fragment 3	pJF751	Bam HI–Pvu II	~3010 BP
Fragment 4	SPV Hind III M	Bgl II–Pst I	~1180 BP



THIS PAGE BLANK (USPTO)

FIGURE 12B

779-94.31
SPV-047

Junction A

[Hind III]

GAA TAC AAG CTA GCT TAA GAA AGA ATG

SPV Hind III M

pSP64

Junction B

[Bgl II, BamH I] Hind III Xba I LP2

GAA TCG TCT AGA TCC CCA AGC TTG GCC TCG AGG GCC GCG GCC GCC TGC AGG TCG ACT CTA GAT TTT

Junction B Continued

LP2 Bgl II EP2

TTT TTT TTT TTT TTT TGG CAT ATA AAT AGA TCT GTA TCC TAA AAT TGA ATT GTA ATT ATC GAT AAT

Junction B Continued

EP2 EcoR I Sma I

AAA TGA ATT CAC CCG CTG GTG GCG GTC TTT GGC GCG GGC CCC GTG GGC ATC GGC CCG GGC ACC ACG

PRV gB (2-16) (Synth tic)

PRV Kpn I C

THIS PAGE BLANK (USPTO)

FIGURE 12C

Junction C

Xba I LP1

GAGC TCG AAT TCG AGC TCG CCC GGG GAT CCT CTA GAA TTT CAT TTT

PRV Kpn I C

Junction C Continued

LP1 EcoR I BamH I

GTT TTT TTC TAT GCT ATA AAT GAA TTC GGA TCC CGT CGT TTT

pJF 751

Junction D

Pvu II

GAA ATC CAG CTG AGC GCC GGT CGC TAC CAT TAC CAG TTG GTC TGG TGT CAA AAA GAT CCA TAA

pJF 751 E. coli Lac Z (1024)

Junction D Continu d

Hind III [Bgl II]

TTA ATT AAC CCG GGT CGA CCT GCA GGG CGG CCG CGG CCC TCG AGG CCA AGC TTG GGG ATC TAA TAA

SPV Hind III M

Junction E

Pst I

TTT TAA AAA TAC GAC TTA CTG CAG GTC GAC TCT AGA

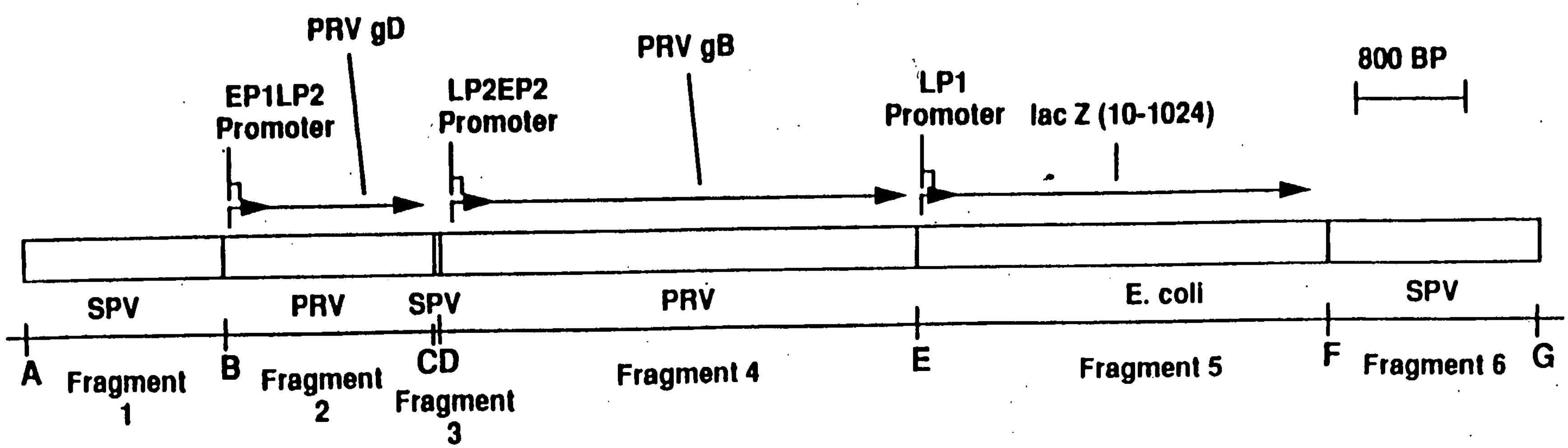
pSP64

SPV Hind III M

THIS PAGE BLANK (USPTO)

FIGURE 13A

DNA	Origin	Sites	Size
Vector	pSP64	Hind III–BamH I	~2972 BP
Fragment 1	SPV Hind III M	Bgl II–Acc I	~1484 BP
Fragment 2	PRV BamHI #7	EcoR I–Stu I	~1552 BP
Fragment 3	SPV Hind III M	Acc I–Nde I	~48 BP
Fragment 4	PRV Kpn I C	Sma I–EcoR I	~3500 BP
Fragment 5	pJF751	Bam HI–Pvu II	~3010 BP
Fragment 6	SPV Hind III M	Nde I–Hind III	~1560 BP



THIS PAGE BLANK (USPTO)

FIGURE 13B

789-41.7
SPV-052

Junction A

[Bgl II, BamH I]

GCC CGG GGA TCT TGA AGA TGA ATG CAT

pSP64 ← → SPV Hind III M

Junction B

Pst I

[Acc I]

EP1LP2

ATT TGG TCT GCT GCA GGT CGA CTC TAG AAA AAA TTG AAA AAC TAT TCT AAT TTA TTG CAC

← SPV Hind III M

Junction B continu d

EP1LP2

EcoRI

GGA GAT CTT TTT TTT TTT TTT TTT TTT TGG CAT ATA AAT GAA TTC GCT CGC AGC GCT

→ PRV Bam HI #7

Junction C

[Stu I]

GCG TGC ACC ACG AGG GAC TCT AGA GGA TCC ATA ATT AAT TAA TTA ATT TTT

← PRV Bam HI #7

Junction C continued

Pst I

[Acc I]

ATC CCG GGT CGA CCT GCA GCC TAC ATG GAA ATC TAC CAG

→ SPV Hind III M

THIS PAGE BLANK (USPTO)

FIGURE 13C

Junction D

Hind III   Xba I

[Nde I]

GCC GTA TCA TAC AAG CTT GGC CTC GAG GGC CGC GGC CGC CTG CAG GTC GAC TCT AGA TTT

SPV Hind III M

Junction D continued

Bgl II

LP2   EP2

TTT TTT TTT TTT TTT TTG GCA TAT AAA TAG ATC TGT ATC CTA AAA TTG AAT TGT AAT TAT CGA

Junction D continued

EcoR I   Sma I

EP2

TAA TAA ATG AAT TCA CCC GCT GGT GGC GGT CTT TGG CGC GGG CCC CGT GGG CAT CGG CCC GGG

PRV gB (2-16) (Synthetic)   PRV Kpn I C

THIS PAGE BLANK (USPTO)

FIGURE 13D

Junction E

EcoR I; Xba I; LP1

GAGC TCG AAT TCG AGC TCG CCC GGG GAT CCT CTA GAA TTT CAT TTT

PRV Kpn I C

Junction E Continued

LP1; EcoR I; BamH I

GTT TTT TTC TAT GCT ATA AAT GAA TTC GGA TCC CGT CGT TTT

pJF 751

Junction F

Pvu II

GAA ATC CAG CTG AGC GCC GGT CGC TAC CAT TAC CAG TTG GTC TGG TGT CAA AAA GAT CCA TAA

pJF 751 **E. coli Lac Z (1024)**

Junction F Continued

Hind III; [Nde I]

TTA ATT AAC CCG GGT CGA CCT GCA GGG CGG CCG CGG CCC TCG AGG CCA AGC TTG TAT GAT AATATC

SPV Hind III M

Junction G

[Hind III]

TAT AAT GGT ATA AAG CTA GCT TGT ATT CTA

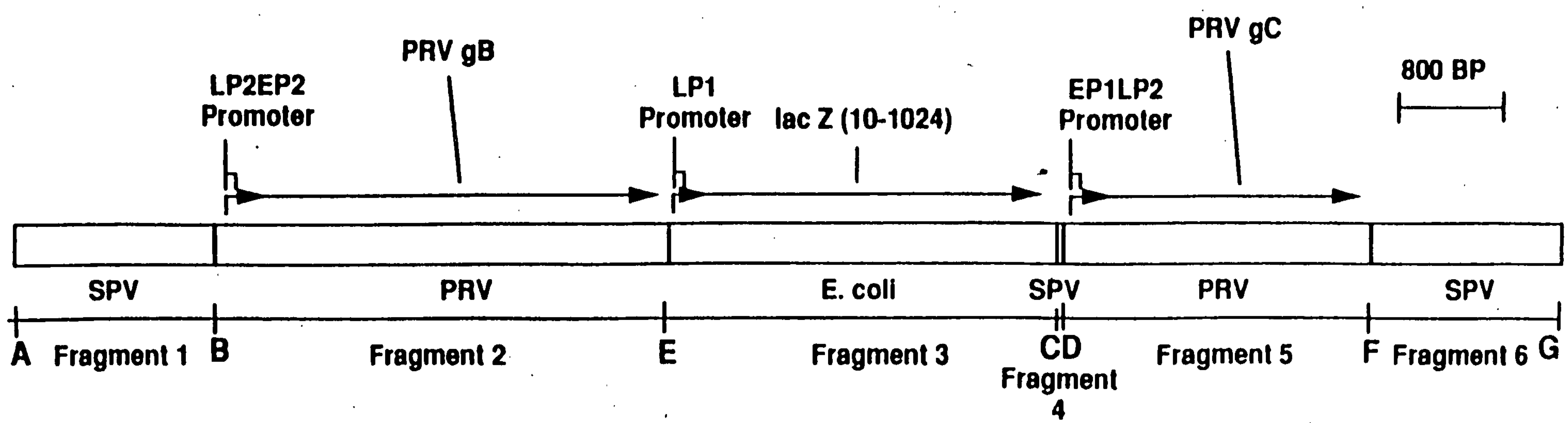
pSP64

SPV Hind III M

THIS PAGE BLANK (USPTO)

FIGURE 14A

DNA	Origin	Sites	Size
Vector	pSP64	Hind III–BamH I	~2972 BP
Fragment 1	SPV Hind III M	Hind III–Nde I	~1560 BP
Fragment 2	PRV Kpn I C	Sma I–EcoR I	~3500 BP
Fragment 3	pJF751	Bam HI–Pvu II	~3010 BP
Fragment 4	SPV Hind III M	Nde I–Acc I	~48 BP
Fragment 5	PRV BamH I #2 & #9	Nco I–Nco I	~2378 BP
Fragment 6	SPV Hind III M	Acc I–Bgl II	~1484 BP



THIS PAGE BLANK (USPTO)

789-41.27
SPV-053

FIGURE 14B

Junction A

[Hind III]

GAA TAC AAG CTA GCT TTA TAC CAT TAT

pSP64 ← | → SPV Hind III M

Junction B

[Nde I] Hind III Xba I

ATA TTA TCA TAC AAG CTT GGC CTC GAG GGC CGC GGC CGC CTG CAG GTC GAC TCT AGA TTT

SPV Hind III M ←

Junction B continu d

LP2 Bgl II EP2

TTT TTT TTT TTT TTT TTG GCA TAT AAA TAG ATC TGT ATC CTA AAA TTG AAT TGT AAT TAT CGA

Junction B continued

EP2 EcoR I Sma I

TAA TAA ATG AAT TCA CCC GCT GGT GGC GGT CTT TGG CGC GGG CCC CGT GGG CAT CGG CCC GGG

→ PRV gB (2-16) (Synthetic)

→ PRV Kpn I C

THIS PAGE BLANK (USPTO)

FIGURE 14C

EcoR I, Xba I, LP1

Junction C GAGC TCG AAT TCG AGC TCG CCC GGG GAT CCT CTA GAA TTT CAT TTT

PRV Kpn I C

LP1, EcoR I, BamH I

Junction C Continu d GTT TTT TTC TAT GCT ATA AAT GAA TTC GGA TCC CGT CGT TTT

pJF 751

Junction D

Pvu II

GAA ATC CAG CTG AGC GCC GGT CGC TAC CAT TAC CAG TTG GTC TGG TGT CAA AAA GAT CCA TAA

pJF 751, E. coli Lac Z (1024)

Junction D Continued

Hind III, [Nde I]

TTA ATT AAC CCG GGT CGA CCT GCA GGG CGG CCG CGG CCC TCG AGG CCA AGC TTG TAT GAT ACG GCA

SPV Hind III M

THIS PAGE BLANK (USPTO)

FIGURE 14D

Junction E

Pst I — Xba I — EP1LP2 — Bgl II

[Acc I]

TGT AGG CTG CAG GTC GAC TCT AGA AAA AAT TGA AAA ACT ATT CTA ATT TAT TGC ACG GAG ATC

SPV Hind III M

Junction E Continued

EP1LP2 — EcoR I — [Nco I]

TTT TTT TTT TTT TTT TTT TTT GGC ATA TAA ATG AAT TCC GGC ATG GCC TCG CTC GCG CGT

PRV BamH I #2 & #9

Junction F

[Nco I]

CCA TGC TCT AGA GGA TCC CCG GGC GAG CTC GAA TTC GGA TCC ATA ATT AAT TAA TTA

PRV BamH I #2 & #9

Junction F Continued

Pst I — [Acc I]

ATT TTT ATC CCG GGT CGA CCT GCA GCA GAC CAA

SPV Hind III M

Junction G

[Bgl II, BamH I]

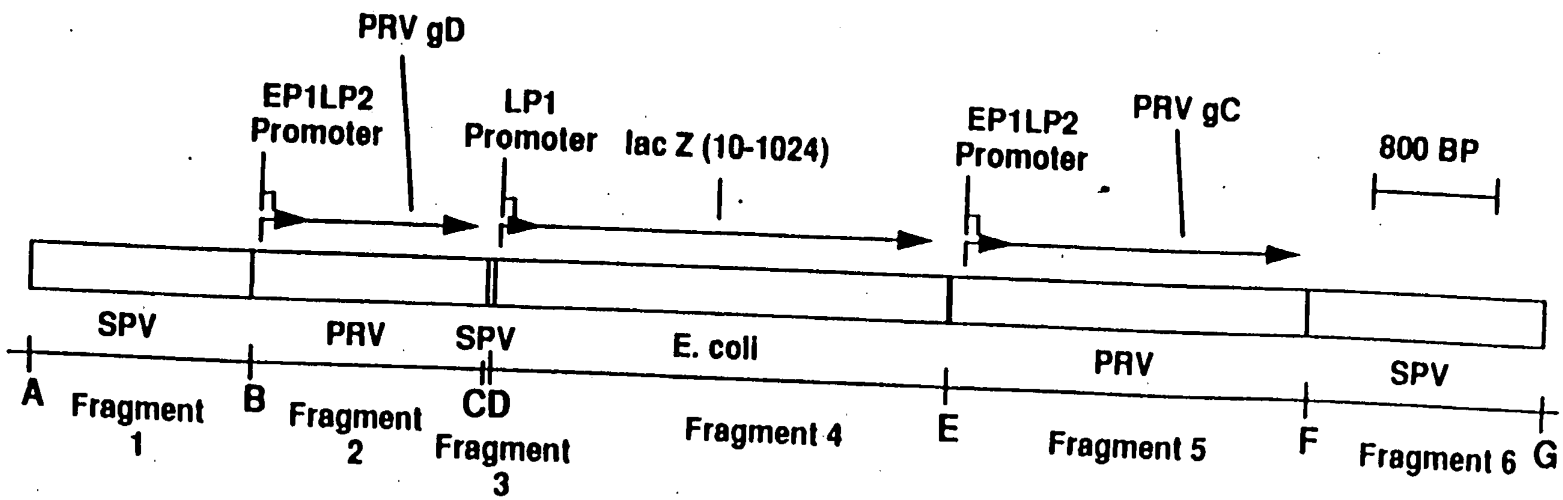
CAT TCA TCT TCA AGA TCC CCG GGC GAG CTC GAA TTC

SPV Hind III M

pSP 64

FIGURE 15A

DNA	Origin	Sites	Size
Vector	pSP64	Hind III–BamH I	~2972 BP
Fragment 1	SPV Hind III M	Bgl II–Acc I	~1484 BP
Fragment 2	PRV BamHI #7	EcoR I–Stu I	~1552 BP
Fragment 3	SPV Hind III M	Acc I–Nde I	~48 BP
Fragment 4	pJF751	Bam HI–Pvu II	~3010 BP
Fragment 5	PRV Bam H I #2&9	Nco I–Nco I	~2378 BP
Fragment 6	SPV Hind III M	Nde I–Hind III	~1560 BP



THIS PAGE BLANK (USPTO)

789-41.47
SPV-054

FIGURE 15B

Junction A

[Bgl II, BamH I]

GCC CGG GGA TCT TGA AGA TGA ATG CAT

SPV Hind III M

pSP64

Junction B

Pst I

[Acc I]

EP1LP2

ATT TGG TCT GCT GCA GGT CGA CTC TAG AAA AAA TTG AAA AAC TAT TCT AAT TTA TTG CAC

SPV Hind III M

Junction B continued

EP1LP2

EcoR I

GGA GAT CTT TTT TTT TTT TTT TTT TTT TGG CAT ATA AAT GAA TTC GCT CGC AGC GCT

PRV Bam HI #7

Junction C

[Stu I]

GCG TGC ACC ACG AGG GAC TCT AGA GGA TCC ATA ATT AAT TAA TTA ATT TTT

PRV Bam HI #7

Junction C continued

Pst I

[Acc I]

ATC CCG GGT CGA CCT GCA GCC TAC ATG GAA ATC TAC CAG

SPV Hind III M

THIS PAGE BLANK (USPTO)

FIGURE 15C

Junction D

Hind III [Nde I] Xba I LP1

GCC GTA TCA TAC AAG CTT GGC CTC GAG GGC CGC GGC CGC CTG CAG GTC GAC TCT AGA ATT TCA TTTT

SPV Hind III M

Junction D continued

LP1 EcoR I BamH I

GTT TTT TTC TAT GCT ATA AAT GAA TTC GGA TCC CGT CGT TTT

pJF 751

Junction E Pvu II

GAA ATC CAG CTG AGC GCC GGT CGC TAC CAT TAC CAG TTG GTC TGG TGT CAA AAA GAT CCA TAA

pJF 751 E. coli Lac Z (1024)

Junction E continu d

Xba I EP1LP2

TTA ATT AAC CCG GTC GAC TCT AGA AAA AAT TGA AAA ACT ATT CTA ATT TAT

Junction E continued

Bgl II EP1LP2 EcoR I [Nco I]

TGC ACG GAG ATC TTT TTT TTT TTT TTT TTT TTT GGC ATA TAA ATG AAT TCC GGC ATG GCC TCG

PRV BamH I #2 & #9

THIS PAGE BLANK (USPTO)

FIGURE 15D

Junction F

[Nco I]

CCA TGC TCT AGA GGA TCC CCG GGC GAG CTC GAA TTC GGA TCC ATA ATT AAT TAA TTA

PRV BamH I #2 & #9

Junction F continu d ATT TTT ATC CCG GGT CGA CCG GGT CGA CCT GCA GGG CGG CCG CGG CCC

Hind III

[Nde I]

Junction F continued TCG AGG CCA AGC TTG TAT GAT AAT ATC AAA

SPV Hind III M

[Hind III]

Junction G TAT AAT GGT ATA AAG CTA GCT TGT ATT CTA

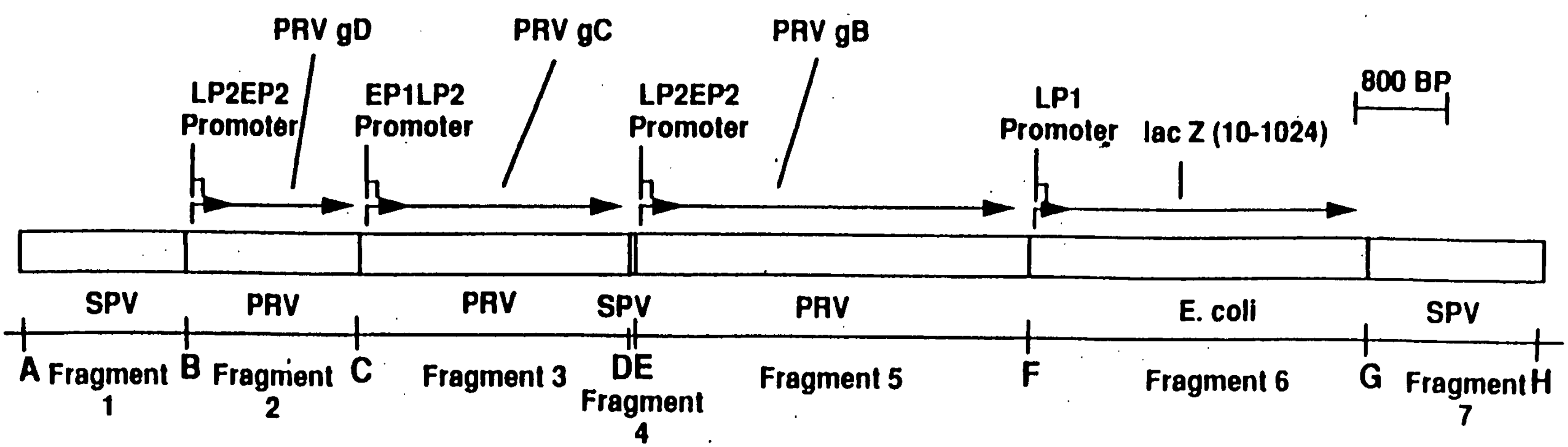
pSP64

SPV Hind III M

THIS PAGE BLANK (USPTO)

FIGURE16A

DNA	Origin	Sites	Size
Vector	pSP64	Hind III–BamH I	~2972 BP
Fragment 1	SPV Hind III M	Bgl II–Acc I	~1484 BP
Fragment 2	PRV BamHI #7	EcoR I–Stu I	~1552 BP
Fragment 3	PRV BamHI #2&#9	Nco I–Nco I	~2378 BP
Fragment 4	SPV Hind III M	Acc I–Nde I	~48 BP
Fragment 5	PRV Kpn I C	Sma I–EcoR I	~3500 BP
Fragment 6	pJF751	Bam HI–Pvu II	~3010 BP
Fragment 7	SPV Hind III M	Nde I–Hind III	~1560 BP



THIS PAGE BLANK (USPTO)

789-41.73
SPV-055

FIGURE 16B

Junction A

[Bgl II, BamH I]

GCC CGG GGA TCT TGA AGA TGA ATG CAT

pSP64 ← → SPV Hind III M

Junction B

[Acc I] Pst I LP2EP2

ATT TGG TCT GCT GCA GGT CGA CTC TAG ATT TTT TTT TTT TTT TTT TTT GGC ATA TAA ATA

← SPV Hind III M

Junction B
continu d

LP2EP2 EcoR I

GAT CTG TAT CCT AAA ATT GAA TTG TAA TTA TCG ATA ATA AAT GAA TTC GCT CGC AGC GCT

→ PRV Bam HI #7

FIGURE 16C

Junction C

EcoR I · Xba I · LP1

GAGC TCG AAT TCG AGC TCG CCC GGG GAT CCT CTA GAA TTT CAT TTT

PRV Kpn I C

Junction C Continued

LP1 · EcoR I · BamH I

GTT TTT TTC TAT GCT ATA AAT GAA TTC GGA TCC CGT CGT TTT

pJF 751

Junction D

Pvu II

GAA ATC CAG CTG AGC GCC GGT CGC TAC CAT TAC CAG TTG GTC TGG TGT CAA AAA GAT CCA TAA

pJF 751 · E. coli Lac Z (1024)

Junction D Continued

Hind III · [Nde I]

TTA ATT AAC CCG GGT CGA CCT GCA GGG CGG CCG CGG CCC TCG AGG CCA AGC TTG TAT GAT ACG GCA

SPV Hind III M

THIS PAGE BLANK (USPTO)

FIGURE 16D

Junction E

[Nde I] Hind III Xba I

GCC GTA TCA TAC AAG CTT GGC CTC GAG GGC CGC GGC CGC CTG CAG GTC GAC TCT AGA TTT

SPV Hind III M

Junction E continued

LP2 Bgl II EP2

TTT TTT TTT TTT TTT TTG GCA TAT AAA TAG ATC TGT ATC CTA AAA TTG AAT TGT AAT TAT CGA

Junction E continu d

EP2 EcoR I Sma I

TAA TAA ATG AAT TCA CCC GCT GGT GGC GGT CTT TGG CGC GGG CCC CGT GGG CAT CGG CCC GGG

PRV gB (2-16) (Synthetic)

PRV Kpn I C

THIS PAGE BLANK (USPTO)

FIGURE 16E

Junction F

EcoR I, Xba I, LP1

```
GAGC TCG AAT TCG AGC TCG CCC GGG GAT CCT CTA GAA TTT CAT TTT
```

PRV Kpn I C

Junction F Continued

LP1, EcoR I, BamH I

```
GTT TTT TTC TAT GCT ATA AAT GAA TTC GGA TCC CGT CGT TTT
```

pJF 751

Junction G

Pvu II

```
GAA ATC CAG CTG AGC GCC GGT CGC TAC CAT TAC CAG TTG GTC TGG TGT CAA AAA GAT CCA TAA
```

pJF 751, E. coli Lac Z (1024)

Junction G Continu d

Hind III, [Nde I]

```
TTA ATT AAC CCG GGT CGA CCT GCA GGG CGG CCG CGG CCC TCG AGG CCA AGC TTG TAT GAT AAT ATC
```

SPV Hind III M

Junction H

[Hind III]

```
TAT AAT GGT ATA AAG CTA GCT TGT ATT CTA
```

pSP64

SPV Hind III M

THIS PAGE BLANK (USPTO)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/12212

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12N 7/01, 15/86; A61K 39/275, 39/295
US CL : 435/235.1, 320.1; 424/199.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/235.1, 320.1; 424/199.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, WPIDS, MEDLINE, CAB. Search terms: swinepox, swine pox?, suipox?, interferon, cytokines, cytokine, interluk?, bovine, recombinant, vector?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,382,425 A (COCHRAN et al) 17 January 1995, see entire document, especially claims 1-20.	1-35
Y	RAMSHAW et al. Cytokine expression by recombinant viruses-a new vaccine strategey. TIBTECH. December 1992, Vol. 10, pages 424-426, see entire document.	11, 21
Y	MASSUNG et al. The Molecular Biology of Swinepox Virus. Virology. 1991, Vol. 180, pages 347-354, see entire document.	1-7, 12, 15, 21, 34, 35

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 SEPTEMBER 1997	28 OCT 1997

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
ALI R. SALIMI
Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/12212

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MASSUNG et al. DNA Sequence Analysis of Conserved and Unique Regions of Swinepox Virus:Identification of Genetic Elements Supporting Phenotypic Observations Including a Novel G protein-Coupled Receptor Homologue. Virology. 1993, Vol. 19, pages 511-528, see entire document.	1-7, 12, 15, 21, 34, 35
Y	FOLEY et al. Recombinant DNA Technology I. Annals of The New York Academy of Sciences. New York, New York: The New York Academy of Sciences. 1991, Vol. 646, pages 220-222, see entire document, especially Abstract, and Results.	34-35